The Impact of Wood Biochar as a Soil Amendment in Aerobic Rice Systems of the Brazilian Savannah

by

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Abstract

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Rice is a staple food for 3 billion people in the world. In Brazil, rice is a traditional staple food mostly cultivated by smallholder farmers. Rice is better adapted to soil types and climate conditions of the Brazilian tropical Savannah than crops like corn and soybean. However, environmental and socio-economic constraints such as the variable rainfall and the limited access to mineral fertilization is a challenge for sustainable aerobic rice production in Brazil. Yields can vary from 1 to 5 Mg ha^{-1}. In this context, the use of agronomic techniques able to improve soil properties seems a good option to increase quantity and stability of rice production. The use of biochar as a soil amendment represents one such option. Biochar is carbonized biomass, generally a by-product of bioenergy production from biomass. Its use in agricultural soils is inspired by the very fertile *Terra Preta* soils, which are a result of pre-Columbian human activity in the Amazon region. A key component of the fertility of *Terra Preta* soils is the high content of C, mostly present in form of pyrogenic C, result of carbonization of organic material. Pyrogenic C is also an important fraction of the soil organic matter present in the weathered soils of the Brazilian Savannah. These soils are mostly acidic, with low soil organic matter content, requiring liming and mineral fertilization if used for agriculture. The biochar tested in the current research is a by-product of charcoal production from eucalyptus wood via slow pyrolysis at 400-500 $^{\circ}$C. It is a porous material with a high C content and K, Ca and Mg availability, which make it a potentially suitable soil amendment for the low fertile soils of the Brazilian Savannah. We applied biochar in a sandy and a clay soil type of the Brazilian Central West region, where over 40% of the Brazilian total crop production is located. We investigated whether biochar amendment improves soil chemical and physical properties and how this in turn affects aerobic rice yields along four cropping seasons after a single biochar application. In both soil types, biochar decreased soil acidity up to 3.5 years after its application. On the clay soil, biochar application decreased the soil water retention capacity but increased the soil organic matter content. The effect of biochar on rice yields on the clay soil were either absent, negative or dependent on the amount of mineral N applied, as well as biochar-induced changes in soil properties, particularly soil water retention and soil organic matter. Most promising results were observed on the sandy soil, where biochar application increased the soil water retention capacity. On the sandy soil, first two seasons were drier than latter two seasons. Accordingly, effects of biochar on rice yields were divergent: the positive effects observed in the first two seasons were absent in subsequent seasons. During this study, weather conditions and rice blast infestations were factors that influenced the observed effects of biochar on rice yields. Further, biochar did not enhance N_2O emissions on the clay soil. Based on these results wood biochar could be considered for use in farming systems of the Brazilian Savannah, particularly on sandy soils.

Keywords: tropical Savannah, biochar, soil fertility, aerobic rice, grain yield, N_2O emission

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Chapter 1

General introduction

Márcia Thaís de Melo Carvalho

"Ideas need time to mature. Toil, which seems fruitless can become, for future generations, a prized possession. In the very wreck of a ruined civilization, a new generation may construct a more secure home. To those who are thinking of the future with anxiety, I say that our present duty is clear: to give all to establish justice and truth in this world."

(Prof. Robert Regout, inaugural lecture Nijmegen, 1940, Dutch Jesuit Archives)

This chapter provides general information about the suitability of biochar as a soil amendment in cropping systems, with emphasis on the effects of biochar on chemical and physical soil properties and crop yield. Edaphic and climate characteristics of the Brazilian Savannah and constraints related to aerobic rice production in this region are presented. Advances in biochar research and the remaining knowledge gaps, which formed the motive for the current study, are discussed.

1. Agricultural profile and aerobic rice production in Brazil

Brazil is the fifth largest country in the world with an area of 851 million ha, of which almost 50% is covered by *Amazônia*, the Amazon Forest (MMA 2006, Fig. 1 - a). The current population is 203 million people and is expected to increase to 223 million by 2030 (IBGE 2014a). Around 30% of the Brazilian territory is occupied by crops (77 million ha) and pastures (173 million ha) (Brazil 2010). Just over 40% of total crop production of the country is located in the tropical Savannah, with the states of *Mato Grosso* (24%) and *Goiás* (10%) as the main producers (IBGE 2014b). The Brazilian tropical Savannah, or *Cerrado*, is mostly situated in the Central West region (Fig. 1 - b). It is a drought prone environment, classified as Aw according to the Köppen-Geiger climate type map of South America (Peel et al. 2007). Ferralsols, acidic and deeply weathered soils, cover 46% of the Savannah region (Battle-Bayer et al. 2010) and 39% of the Brazilian territory (Embrapa 1999, Fig. 2 - a). The region is characterized by two well-defined seasons: a dry (from April to August) and a wet season (from September to March) (Fig. 3).

Agriculture expansion in the *Cerrado* occurred from the 70’s to the 90’s as a consequence of governmental policies, investments in scientific research and pioneer farming. Technologies such as liming and rice and soybean varieties adapted to the local circumstances were instrumental for development of agriculture in the Savannah biome. In recent years, the agricultural expansion in the Brazilian *Cerrado* received worldwide recognition (Tollefson 2010, The Economist 2010). Agriculture now occupies ~50% of the biome’s original extent and the pronounced conversion of the *Cerrado* into soybean monoculture and soybean/maize succession over the past two decades was one of the main contributors to the expansion in total cropland area in Brazil (Lapola et al. 2013).

Rice was a pioneer crop in this region. Farmers mainly used tropical japonica varieties adapted to rain fed conditions and tolerant to soil acidity (Ferreira et al. 2005). Rice grown in a)

b)

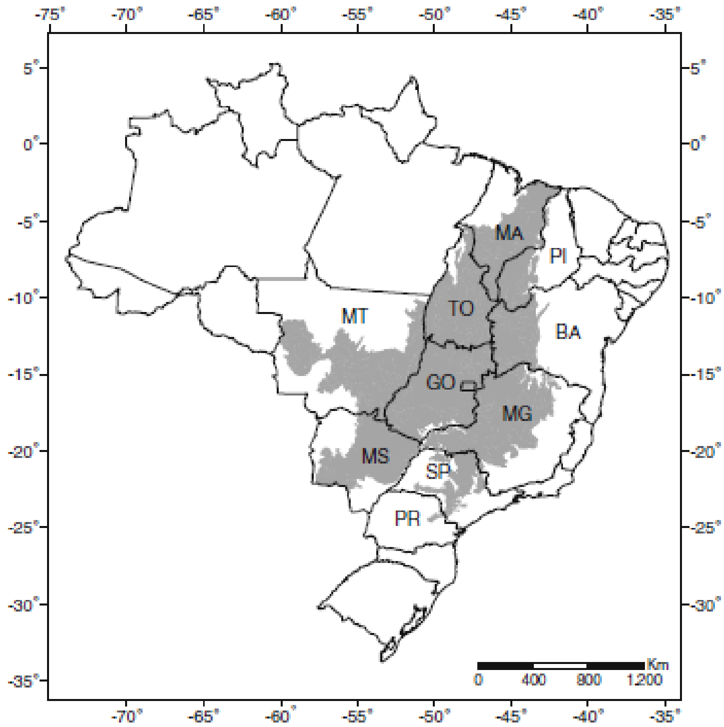
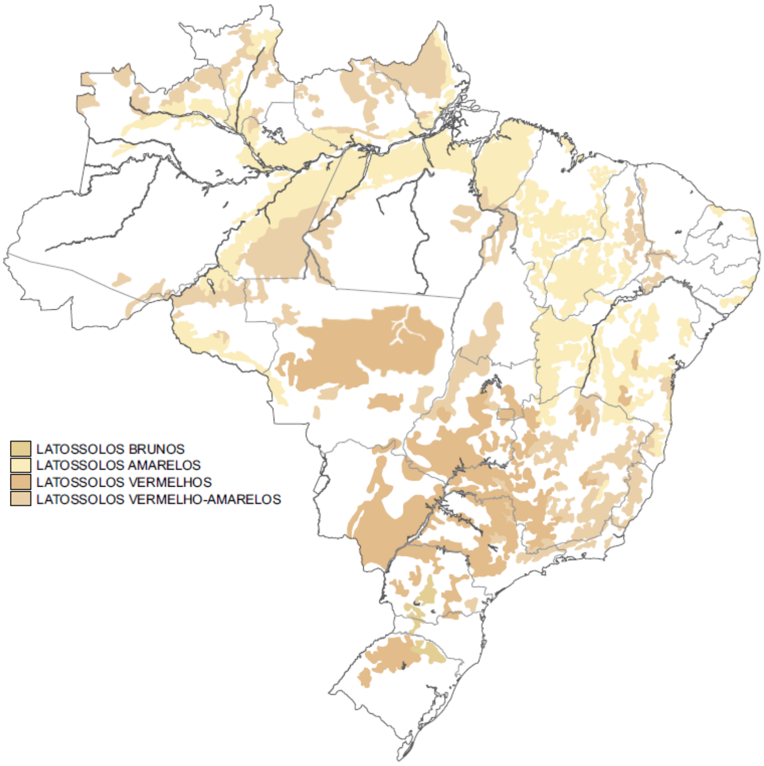


Figure 1. Brazilian morfoclimatic domains (a) and the area covered by the Savannah biome (*Cerrado*) in sates of Brazil: Maranhão (MA), Piauí (PI), Bahia (BA), Tocantins (TO), Goiás (GO), Mato Grosso (MT), Mato Grosso do Sul (MS), São Paulo (SP), Paraná (PR) and Minas Gerais (MG) (b). Source: Ab’Sáber (2000) (a); and Sano et al. (2010) (b).

rain-fed, naturally well-drained soils, without surface water accumulation is called upland or aerobic rice (Fageria 2001). Changing the aeration status of the soil has significant consequences for dynamics, movement and availability of nutrients to plants. Of particular relevance for nutrient dynamics in aerobic systems is soil mineral N availability, as a consequence of alternating cycles of nitrification and denitrification as described by Gaydon et al. (2012) and observed by Massod et al. (2014).

In 1974, around 80% of the rice harvested in Brazil was from aerobic systems (Pinheiro et al. 2006). In the middle 80's, rice was the main crop in the Central West region, covering 2.7 million ha. From the beginning 1980 to 2013, however, the area cultivated with rice in this region declined from 2.7 to 0.2 million ha (Conab 2014a). A remarkable decrease started at the beginning of the 90's, mainly due to the growing interest in more profitable crops, such as soybean and the adoption of a breeding program strategy focused on selection of plants for most favorable areas (Pinheiro et al., 2006). This breeding strategy lead to a high risk of developing genotypes specialized and susceptive to crop failure due to dry spells. Dry spells with a duration of 5 to 7 consecutive days frequently occur in January and February, in the middle of the wet season. Regional climate risks zoning for aerobic rice in the Central West region of Brazil indicates that best sowing dates are from October to December (Silva and Assad 2001). Further, Heinemann and Sentelhas (2011) proposed an environmental group identification to guide breeding programs focused on adaptation of aerobic rice production to less favourable, favourable and highly favourable conditions in Central Brazil. Supplementary sprinkler irrigation can also alleviate negative consequences of dry periods along crucial stages of the rice-growing season (Crusciol et al. 2013).

Around 18% of the global surface water and 1% of the underground water is in Brazilian territory (Almeida et al. 2014). From the total amount of surface water consumed in Brazil, 69% is utilized in irrigated agriculture, with an average efficiency of 64% (MIN 2008). In 1970, the total irrigated area in Brazil was around 2.3% of the total agricultural land, and further increased to 6.0% in 1995 and 8.3% in 2012 (ANA 2013). Yet, the water used for irrigation is not charged in most of the Brazilian states. Although new technologies, modern equipment and specialized information is available, the management of irrigation and the rational use of water still needs investments for additional optimization. Most of the issues regarding the use of irrigation in agriculture is related to required investments in infrastructure, and the poor water distribution in the country (around 70% of the surface water available is located in Amazon, where only 7% of the population lives, Fig. 2 - b). The state a)



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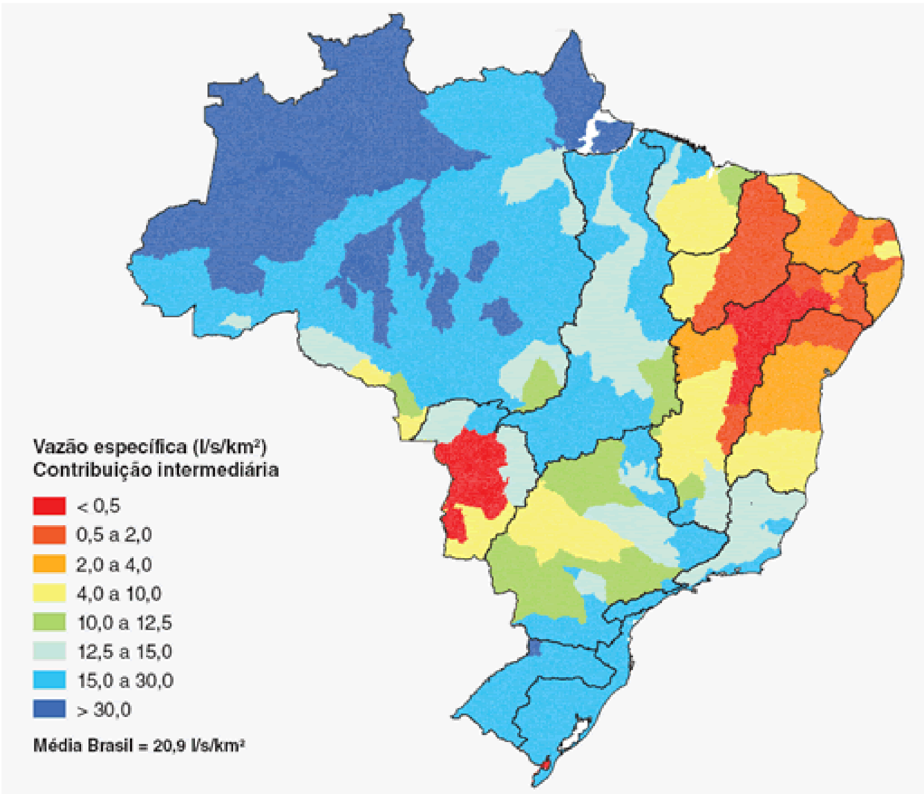

Figure 2. Distribution of Ferralsols (a) and distribution of superficial water in l/s/km^2 by hydrographic region (b) on the Brazilian territory. Source: IBGE, 2007 (a); and ANA, 2009 (b).

of São Paulo in Southeast Brazil, for example, is currently passing through a historical water crisis and is expected to have a gap of 14% between demand and supply of water by 2030 (Addams et al. 2009). Of all sectors of the economy, agriculture is the most sensitive to water scarcity. Accounting for 70% of global water withdrawals, it is also the sector where intelligent adjustments offer the largest scope for significant water savings (FAO 2012).

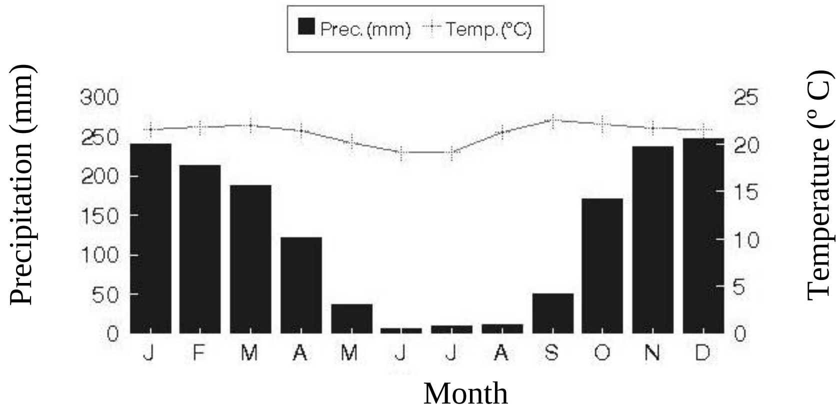

Figure 3. Climate map of Brasília-DF (15° 46′ 48″ S, 47° 55′ 45″ W) for the period from 1963 to 1990. Typical climate map in the Brazilian tropical Savannah. Source: http://www.inmet.gov.br/.

Flooded systems represent around 80% of total rice production in Brazil, mostly located in South, with an average yield of 7 Mg ha^{-1} and occupying 51% of the total area cultivated with rice. The Central West represents the second largest rice-producing region, mostly on aerobic systems, with an average yield of 3 Mg ha^{-1} (Silva and Wander 2014). The current total area of rice grown in Brazil is about 2.4 million ha of which 10% is located in the Central West region (Conab 2014a). Although the area of aerobic rice in Brazil has decreased, productivity has increased by 0.67% per year (~ 19 kg ha^{-1} $year^{-1}$) mainly due to genetic improvements in the period from 1984 to 2009 (Breseghello et al. 2011). In recent decades, the productivity growth for several crops, including rice, has allowed Brazil to become one of the world's largest agricultural exporters (Foresight 2011, The Economist 2010). Soybean is the most cultivated crop in Brazil, covering 42% of total grain area, followed by corn (38%) (Brazil 2010). Main export products are soybean and by-products (27%), meat (18%), sugar and ethanol (15%), and coffee (7%). Main destinations of these products are the European Union (29%), China (14%), USA (7%) and Russia (4%). Rice (including lowland, flooded and aerobic systems) represents only 9% of the total area cultivated with crops in Brazil and it represents 4% of the gross value of the major agricultural production (Brazil 2010).

Smallholder farming provides about 34% of the rice consumed in Brazil (IBGE 2006). Smallholder farming or family farming is characterized by its structure of labour and source of income: the majority of labour used in on-farm economic activities has to be provided by the family, and the size of the property cannot exceed 4 fiscal modules or 400 ha (Diniz 2013).

Rice is not amongst the list of major exports. In fact, Brazil imports rice to keep up with domestic demand. In 2014, Brazil imported almost 130,000 Mg, mainly from Uruguay, Paraguay and Argentina (Conab 2014b). Yet, Brazil is the ninth world rice producer, harvesting 12 million Mg in 2012 (FAO STAT 2014). Systematic cultivation of rice (*Oryza sativa*) in Brazil was established by the Portuguese around 1766. Yet there is evidence that native wild species of rice (*Oryza subulata*, *Oryza caudata*) were consumed and known by indigenous people as *abatipê* in Amazon lakes and *abati-mirim* in the lowlands of the Savannah (Cascudo 2011, Fig. 4). Nowadays it is known that four wild species of rice (genus *Oryza*) occurs in Brazil: *O. glumaepatula*, *O. latifolia*, *O. alta* and *O. grandiglumis* (Rangel et al. 2005). Domestic consumption of rice nowadays is about 160 g per person per day, feeding around 84% of the Brazilians, who rely on rice as their main daily source of carbohydrate (IBGE 2011). The access to safe and stable regional and international sources and more productive and adapted cropping systems can guarantee the country's supply of this staple food in a sustainable manner. One of the big challenges in Brazil is to achieve food security. In 2014, *The State of Food Insecurity in the World'* report (FAO, IFAD and WFP, 2014) revealed for the first time that Brazil achieved the targets of halving the proportion of its people who suffer from hunger and of reducing the absolute number of hungry people by half. Overall, the proportion of undernourished people fell from 10.7% of the population in 2000/02 to less than 5.0% in 2004/06. Investments in family farming have had a significant impact. Over the last ten years, family farmers' income has increased by 52% in real terms.

Nowadays, the main objective of the research on aerobic rice in Brazil is to include rice in more integrated systems, including rotation with other crops, such as soybean and maize, and pasture under no-tillage systems with supplementary irrigation (Embrapa, 2003). Under these conditions, aerobic rice has a reasonable performance and can favour the performance of other crops in succession. The use of legume species or cover crops in rotation or intercropping systems is also an alternative to improve aerobic rice yields in a tropical Savannah (Akanvou et al. 2002, Nascente et al. 2013a). Nevertheless, efficient use of irrigation, improvement of soil water retention capacity and an integrated control of pests and

Figure 4. From the book *História da Alimentação no Brasil* by Cascudo (2001) about the establishment of rice as a stable food in Brazil: “It was not, among us, food for slaves nor sustenance for travellers. Alone, it did not gorged no one, like dry farina of cassava or cooked corn. The cultivation was not easy. The plantation on lowlands required attention and caution. The ‘dry’ types were more threatened by parasites plagues. It was always known, but never sought. It is eaten by habit, custom, and tradition. It is obvious that the industrial technics and the culinary refinement found in rice raw material for income and surprises.”

disease are important challenges for increasing productivity and sustainability in aerobic rice systems. Therefore, there is considerable scope to increase the productivity of aerobic rice systems in Brazil. Aerobic rice yields are highly variable, ranging from 1 to 5 Mg ha^{-1} (Conab 2012a). Besides weeds and rice blast infestations, rainfall variability is one of the main limiting factors for aerobic rice production in the Savannah region (Heinemann and Sentelhas 2011). Rice (*Oryza sativa* L.) is a “water loving plant” (Prasad 2011). A minimum water supply of 400 to 600 mm, well distributed along the entire growing season, is required in order to avoid yield losses in aerobic rice systems (Crusciol et al. 2013). Moreover, the acidity of Ferralsols in the Savannah leads to restricted rooting depth, which promulgate water stress during dry spells. Especially around the reproductive phase, water stress can cause poor grain filling. Around 220 to 250 mm of rainfall between panicle initiation and flowering stages is necessary to avoid high number of sterile spikelets and guarantee a reasonably successful harvest (Pinto and Assad 2008). Next to the development of improved rice varieties, improvement of soil water retention is an important agronomic strategy to improve rice productivity and advance towards more adapted and productive rain fed rice systems in the

Cerrado. Evidently, any option for improving the water holding capacity in aerobic rice production areas should be embraced. One interesting option that might contribute to realize such an improvement is the use of carbonized biomass or biochar as soil amendment. The main question addressed in the research described in this thesis is whether biochar can provide the desired improvements of soil properties in the weathered and acidic soils of the *Cerrado*, leading to positive effects on crop production.

2. Biochar definition

Biochar is simply charcoal, a porous, black solid by-product of carbonized biomass, obtained via pyrolysis in the absence of air (Sohi et al. 2010). It is a versatile material with potential applications in agriculture (Lehmann and Joseph 2009, Schmidt 2012). The difference between biochar and mineral char is that biochar can be produced from many different available sources of organic material whereas mineral char (or coal: mineral of fossilized carbon) is a combustible black sedimentary rock extracted from underground. Sohi et al. (2009) categorized a variety of by-products, including biochar, according to the biotic or abiotic processes applied to transform animal residue, domestic waste or biomass into bioenergy, biochemicals or soil amendment (Fig. 5). The term 'bio' has an environmentally friendly connotation and this is no coincidence. Burning mineral char for energy production leads to a net addition of CO_2 to the atmosphere, thus accelerating climate change induced by greenhouse gases emission. Conversely, the use of biomass for energy production is 'CO_2 neutral', i.e., the CO_2 is fixed in the organic material via photosynthesis and can return to atmosphere via pyrolysis. If the pyrolysis is incomplete, then small biochar particles remain, and if gases result of pyrolysis are captured to produce more energy, then a small part or zero CO_2 returns to the atmosphere, in which case there is a net extraction of CO_2 from the atmosphere, closing the cycle (Lehmann 2007a).

In Brazil, demand for energy produced from charcoal has increased substantially in the last decades. As a result, the area covered with timber plantations specifically for charcoal production has also increased. In 2010, 35% of wood from forest plantations was destined to charcoal production (Abraf 2011). The expansion of the wood sector can reduce the pressure on native forests and empower Brazil to mitigate greenhouse gas emissions (Uhlig et al. 2008). In 2005, the land use change sector was responsible for ~ 60% of the total Brazilian CO_2 emissions. However, with the pronounced countrywide reduction in deforestation, there was a reduction (~10% from 2005 to 2010) in total emissions. Consequently, agriculture has become the leading greenhouse gas emitter, representing 37% of all Brazilian emissions (MCTI 2013).

The feedstock and production process applied for the biochar used in this thesis is shown by the highlighted boxes in Figure 5. The feedstock was eucalyptus wood, which is used for charcoal production for iron smelters. In this process, the biochar remains as residue. If the biochar pieces are large enough, they can be used for domestic cooking and barbequing. Smaller pieces of biochar are unsuitable for cooking but may be used as a soil amendment. Figure 6 shows this type of biochar and the manual process of spreading it out over the soil before incorporation. In the following sections, we review the current state of knowledge on biochar as a soil amendment and discuss its effects on soil, plant and atmosphere.

3. Research on biochar and crop production

According to Lehmann and Joseph (2009) biochar can be considered an important tool for addressing a wide range of important challenges: soil degradation and food insecurity, climate change, sustainable energy generation and waste management (Fig. 7 - a). The focus of this thesis was on the use of biochar for soil improvement and crop production. In recent years, research on biochar as a soil amendment for enhancement of crop production has increased (Fig. 7 - b). Two developments have sparked particular interest in using biochar. Firstly, the discovery of the Indian Black Earths (*Terra Preta de Indio*) suggests that one of the reasons for the extraordinary fertility of these soils is the high C content (up to 15%), mainly present in the form of pyrogenic C, as the result of ancient human activity in the Amazon (Madari et al. 2003, Novotny et al. 2009). A key process in formation of *Terra Preta* is pyrolysis, which catalyses transformation of organic material, “locking” the C in the form of charcoal. Secondly, the production of bioenergy via carbonization of organic residues or biomass has opened the possibility of amplifying the use of biochar, a by-product in this process (Lehmann 2007b). According to Ogawa and Okimori (2010) the first studies on usefulness of biochar in agriculture/forest soils were conducted in Asia, where intensive agriculture has been practiced since ancient times. In Europe, there are reports on the use of peat-charcoal for agricultural purposes by Davy (1856), and in USA, Tryon (1948) reported on the effect of wood biochar on the properties of forest soils. Recent studies suggest that application of biochar as a soil amendment has potential for increasing crop productivity on weathered tropical soils (Glaser et al. 2002, Lehmann et al. 2003, Steiner et al. 2007, Major et al. 2010,

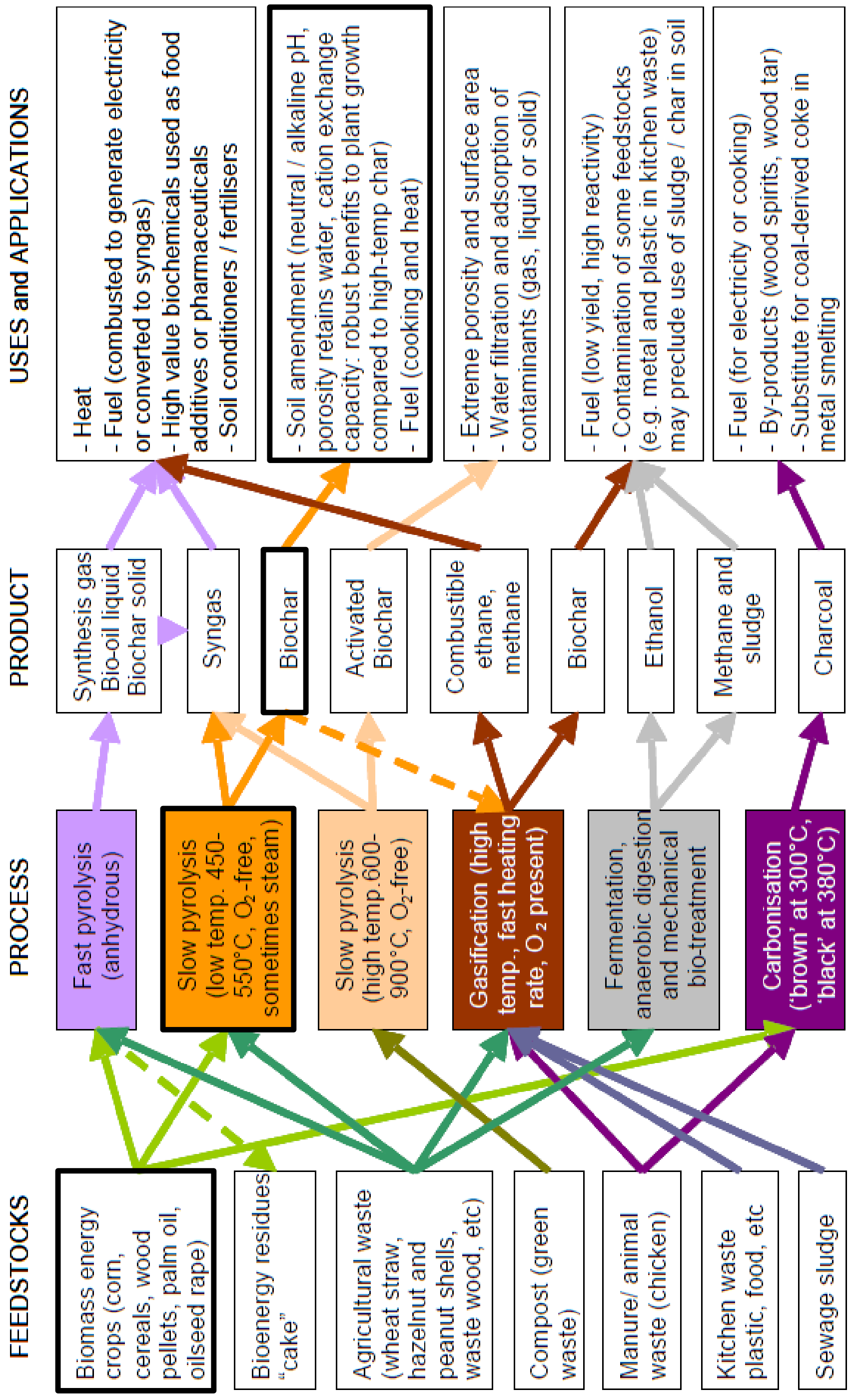

Figure 5. Summary of pyrolysis processes in relation to their common feedstocks, typical products and the applications and uses of these products. Adapted from Sohi et al. (2009).

Petter et al. 2012). This is in agreement with a meta-regression analysis by Crane-Droesch et al. (2013), who found that soil cation exchange capacity and organic C were strong predictors of yield response in the presence of biochar. Soils with low cation exchange capacity and low C were associated with positive crop yield response. Further meta-analysis on the impact of biochar on crop production has shown that generally there is a positive effect of biochar on crop yields (Jeffery et al. 2011, Biederman and Harpole 2013, Liu et al. 2013). However, the extrapolation of these results to field applications is problematic because most studies were conducted over short periods of time and under environmentally-controlled conditions. For example, according to the meta-analysis by Liu et al. (2013), greater responses were found in pot than in field experiments. In addition, Jeffery et al. (2011) showed that effects of biochar on crop yields could vary with crop species without significant effects for rice. Further, it is not always clear whether positive effects stem from a direct fertilization effect, an increased water holding capacity, or a combination of effects.

a) b)

Figure 6. Pieces of wood biochar ≤ 8 mm (a) and application on the clay soil after milled to pass 2 mm sieve (b). Pictures made by B. E. Madari in 08/05/2009 (a) and 09/06/2009 (b).

It is also not well established for how long after biochar application these effects are likely to last in a cropping system. Further, both Jeffery et al. (2011) and Liu et al. (2013) found that crop responses to biochar amendment were greater for dry land crops on acid and sandy textured soils. There is therefore a need for research that addresses the potential of biochar as a soil amendment in aerobic rice production systems under field conditions in a multiple year perspective. The research described in the current thesis focusses on this issue.

4. Determining factors and potential effects of biochar on soil, plant and atmosphere

The International Biochar Intiative (IBI 2013) states that biochar can be used as a product itself or as an ingredient within a blended product, with a range of applications as an agent for soil improvement, improved resource use efficiency, remediation and/or protection against particular environmental pollution, and as an avenue for greenhouse gas abatement. However the large range of biochar types, resulting from different feedstock and pyrolysis processes, leads to a variable efficacy of biochar as soil amendment. For example, the higher the temperature of pyrolysis, the greater the surface area, pH and capacity to exchange cations and the lower the percentage of C recovery (Lehmann 2007b). Biochar types with high ash contents can reduce soil acidity, increase soil pH and concentration of essential elements as Ca, Mg and K and decrease Al availability (Deenik et al. 2011, Deal et al. 2012) while high surface area biochar can improve soil water retention capacity (Gray et al. 2014). Biochar is mainly composed of carbon (C). Major part of this C is resistant (also known as pyrogenic C,

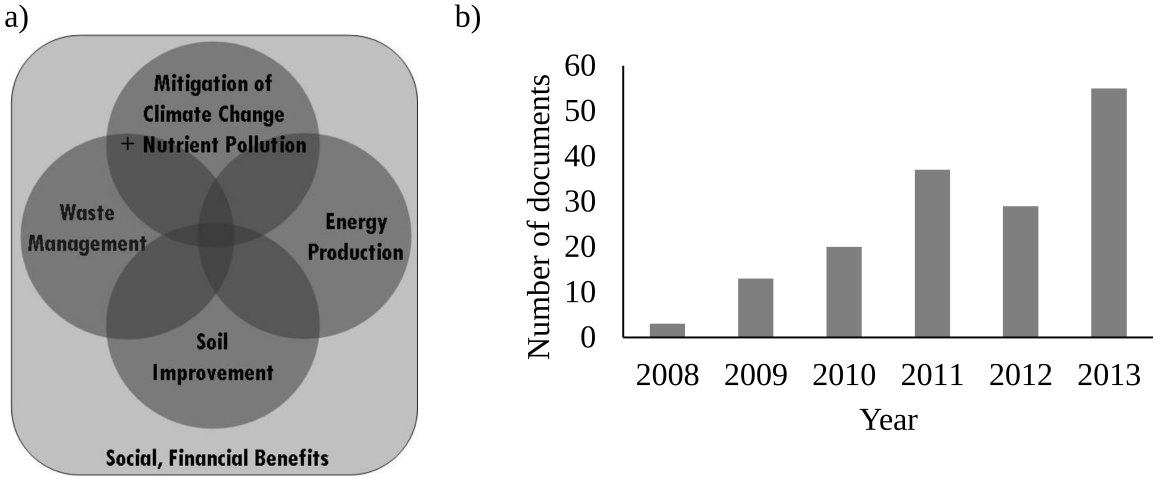

Figure 7. The functionalities of biochar (a) and the number of scientific articles per year for the query ((TITLE-ABS-KEY(biochar and crop production)) (b). Source: Lehmann and Joseph (2009) (a); and Scopus, 157 results, search done on 18 June 2014 (b).

char, black C), and a lesser part is labile, which can affect the soil C/N ratio. In periodically burned open Savannah, fire-derived organic matter is an important component of the soil organic C (SOC) (Novotny et al. 2009). Liming and fertilization effects promoted by the ash can persist for at least 2 years in the *Cerrado* (Pivello et al. 2010). In a topsoil of a 23 years old pasture of the Brazilian Savannah, Roscoe et al. (2001) observed that 50% of the total SOC was resilient due to charred material. Jantalia et al. (2007) estimated that up to 40% of total SOC in a Ferralsol cultivated with soybean based crop rotation system was of pyrogenic origin. The addition of biochar to the soils of the *Cerrado* is likely to build more resilient and fertile aerobic rice cropping systems via improvements of soil organic matter, increased soil pH and capacity to exchange ions and retain water.

Several studies have reported the potential role of biochar in improving soil water retention capacity (Table 1). Table 1 shows that more studies tested the effect of biochar on soil water retention capacity of sandy soils (14) than of clay soils (8) and others (2). The majority of these studies were conducted under artificially controlled conditions (16). A variety of types and a wide range of amounts of biochar have been tested, including wood biochar (16). Overall, most studies applied unfeasibly high amounts of biochar (15 with rate ≥ 2% w/w) rather than more realistic amounts (9 studies with rate ≤ 2% w/w). On sandy soils, almost all studies showed positive effects, whereas on clay soils more often neutral (zero) effects were reported. The positive effects are generally related to the high specific surface area derived from the porous structure of biochar, which behave as additional capillaries, favouring the retention of water by the soil. Yet only 33% of the studies in Table 1 reported this characteristic. This brief review points out that positive effects of biochar on water retention capacity will depend not only on soil type but also on biochar type and rate.

According to Glaser et al. (2002), the physical and chemical composition of slow pyrolysis wood biochar can increase the soil water holding capacity and positively affect soil chemical properties. Additionally, its alkaline pH can decrease soil acidity. If soil acidity decreases, the capacity of soil to exchange ions increases. As a result, biochar can improve nutrient use efficiency. Because wood biochar can improve soil chemical and physical properties, higher yields are expected. Apart from the type of biochar, soil type, crop and amount of biochar applied, the time since incorporation of biochar into the soil is a relevant factor for its effect on soil properties and plant production. Biochar can interact with the soil matrix and go through a weathering process that can change its original physical and chemical characteristics, a process known as "ageing" (Kookana et al. 2011).

Another relevant factor regarding the use of biochar as a soil amendment is its availability. In developed regions of the world, such as in Europe, carbonisation of domestic waste is already a common practice. Biochar can be the link between biomass/residue management and energy/food production. In the Netherlands only, there are 12 waste-to-energy plants treating 7.7 million tons of residues per year (CEWEP 2014) and, according to the Dutch Waste Management Association (2009-2014), the Dutch waste-to-energy plants currently deliver almost 12% of all sustainable energy produced in the Netherlands. A network to study the impact of the use of biochar in agriculture systems is already in place via the European Biochar Research network running from 2012 to 2016. Programs such as the "Interreg IVB project Biochar: climate saving soils" are launching first final reports over the last four years of extensive research into many questions related to biochar production and biochar applications in seven North Sea countries (http://www.biochar-interreg4b.eu/).

The biochar research in Brazil started about 10-15 years ago (Maia et al. 2011). In an

Amazon Ferralsol, Steiner et al. (2007) showed a promising positive effect of wood biochar combined with organic fertilization on aerobic rice production. In the *Cerrado*, the effect of biochar has been studied since 2006 in different soil types (Maia et al. 2011). This thesis is a contribution to the knowledge obtained via these field experiments in the *Cerrado*. Previous pot experiments showed significant effects of wood biochar on decreasing acidity and increasing soil nutrient availability of a clay soil and consequently improving aerobic rice growth and biomass (Madari et al. 2006). Later, Pereira et al. (2012) demonstrated the significant effect of wood biochar on increasing the capacity of sand to hold water. Further, in a field trial Petter et al. (2012) observed positive effects of biochar on soil chemical properties and prominent positive effects on aerobic rice yields on a sandy loam soil immediately and one year after application. The hypotheses of the current study were also established based on these previous results. For instance, the contribution of biochar on improvements of water retention capacity and chemical properties of a sandy soil is probably greater than in a clay soil, which naturally has higher capacity to exchange cations and retain water. Moreover, biochar may have a significant residence time in soils (Schmidt et al. 2011, McHenry 2011), a desirable characteristic under conditions favorable to mineralization such as in tropical Savannahs.

According to Laird et al. (2008), the so-called “win-win-win” scenario with biochar use passes through the bioenergy production, C sequestration, and improvement of soil and water quality. The establishment of this scenario requires establishing the long-term effects of biochar on soil properties under real farming conditions, which depends, as described above, on a multitude of factors. There is also an urgent need to determine the environmental impact of biochar use in a cropping system, such as its effect on nitrogen use efficiency. Biochar potentially has the ability to manipulate the rates of N cycling in soil systems by influencing nitrification rates and adsorption of ammonia and increasing ammonium storage by enhancing cation exchange capacity in soils (Clough and Condron 2010). Its influence on these processes may have further implications in terms of reducing nitrate leaching and gaseous N losses such as N_2O. However, applications of excessive amounts of biochar and mineral N in soil might provide energy in the form of labile C and ammonium for nitrifying bacteria to produce nitrate. Under intermittent aerobic/anaerobic conditions, denitrifying bacteria can transform nitrate into N_2O and N_2. The question is whether high doses of biochar amendment combined with mineral N fertilization can increase rice productivity and decrease N_2O emissions. There are many studies reporting on a potential suppressive effect of biochar on

Reference	Feedstock for biochar production	SSA (m^2 g^{-1})	Rate % (*w/w*)°	Soil type	Set	BD	WRC
Abel S. et al. (2013)	maize, maize silage and beech wood	nd	1, 2.5, 5	sandy and clay	F/L	-	+
Asai et al. (2009)	wood	nd	0.3, 0.6, 1.2*	clay/silt loam	F	nd	0/+
Basso A.S. et al. (2013)	red oak	nd	3, 6	sandy loam	L	-	+
Beck D.A. et al.(2011)	shells and car tire	nd	7	green roof soil	L	nd	+
Brewer C.E. et al. (2012)	corn stove	4.5-8.5	0.5	loamy fine sand	L	nd	0
Brockhoff S.R. et al. (2010)	switch grass	21.6	5, 10, 15, 20, 25	sandy	L	nd	+
Chen Y. et al.(2010)	bagasse of sugarcane and agricultural sewage	nd	3, 1	heavy clay	F	nd	+
Dempster D.N. et al. (2012)	eucalyptus	273	1.8	sandy soil	L	nd	+
Devereux R.C. et al. (2012)	wood	nd	1.5, 2.5, 5	sandy loam	L	-	+
Fellet G. et al. (2011)	prune residues	141	1, 5, 10	clay	L	nd	+
Ibrahim H.M. et al.(2013)	wood	nd	0.5, 1, 1.5, 2	sandy loam	L	nd	+
Jones B.E.H. et al. (2010)	green waste	nd	2.4, 4.6	sandy	L	-	+
Karhu et al. (2011)	hardwood	3.6	0.3	silt loam	F	nd	0
Laird D.A. et al. (2010)	wood	130-153	0.5, 1, 2	fine-loamy	L	-	+
Lei O. & Zhang R.(2013)	dairy manure and woodchip	14-124	5	loamy	L	-	+
Liu J. et al. (2012)	residues of commercial production	nd	0.3, 0.6, 1.2*	loamy sand	F	nd	+
Major J. et al. (2012)	wood	nd	3	clay	F	-	0
Pereira et al. (2012)	wood	nd	6, 12, 24	sandy	L	-	+
Tammeorg P. et al. (2013)	wood	nd	0.4, 0.8	sandy clay	F	0	0
Tryon, E.H. (1948)	pine wood /oak wood	nd	15, 30, 45 †	clay loam /sandy loam	L	nd	-/+
Ulyett J. et al. (2014)	wood	nd	3	sandy loam	L	nd	+
Uzoma K.C. et al. (2011)	cow manure	nd	0.4, 0.7, 0.9	sandy	F	nd	+
Ventura F. et al. (2012)	wood	nd	1.2, 2.4	clay loam	F	-	0
Zheng H. et al. (2013)	grass	2.84	1, 2, 5	silt loam	L	nd	+

SSA: specific surface area of biochar; L: laboratory, artificially controlled conditions; F: field conditions; -: negative effect of biochar; +: positive effect of biochar; 0: no effect; nd: not determined; †: percentages of rate in a volume basis (*v/v*). °All studies have included a control treatment, soil without biochar. *Biochar application rate transformed to a dry mass basis (*w/w*) considering a soil bulk density of 1.3 g cm^{-3} for clay and silt loam soils and 1.6 g cm^{-3} for sandy soils in a soil depth of 10 cm (when not specified).

Table 1. The effect of biochar on soil water retention capacity (WRC) and bulk density (BD) under different soil types and experimental conditions.

N_2O emissions (Lehmann et al. 2006, Sohi et al. 2010, Atkinson et al. 2010, Cayuela et al. 2010, Spokas et al. 2009, Zhang et al. 2010), as well as some evidence of a neutral effect (Scheer et al. 2011, Karhu et al. 2011). Reduction of soil bulk density, increased soil aeration and possible immobilization of N are among the causes for reduced fluxes of N_2O with biochar application. Further, data on the effect of biochar on GHG emissions are contradictory (Mukherjee and Lal 2014). Contradictory results for the effect of biochar amendment on N_2O-N fluxes seems not only related to differences in soil texture and biochar types, but mainly due to variances in soil moisture state. Clearly, the interaction between biochar and mineral N fertilization and its effect on N_2O fluxes in aerobic rice cropping systems needs further attention.

5. Research questions, objectives and thesis outline

The general research question addressed in this study was: *can biochar be used in an agronomically beneficial and sustainable manner to increase the productivity of aerobic rice production systems in a tropical Savannah?* Our perception of "agronomically beneficial and sustainable manner" is further clarified by the following sub-set of specific research questions:

a) What is the effect of a single application of biochar on growth and yield of aerobic rice in the first four cropping seasons after application?
b) Is there an effect of biochar on water holding capacity? Is this effect more prominent on a sandy than on a clay soil, which already has a higher water retention capacity?
c) How much biochar is required to improve soil chemical properties and for how long do these improvements last?
d) Can excessively high doses of biochar reduce N availability to the crop and affect crop growth and yield negatively?
e) Can biochar amendment affect N_2O-N fluxes throughout the growing season and are these changes related to changes in soil chemical and physical properties?
f) What are the overall implications of our findings for the practical use of wood biochar as a soil amendment in aerobic rice cropping systems in a tropical Savannah?

In order to elucidate these research questions the main objective of this study was to determine how wood biochar and mineral N fertilization rates affect aerobic rice yields on a rainfed sandy soil and on an irrigated clay soil in the Brazilian Savannah. To this purpose, field experiments with a single wood biochar application were maintained up to 3.5 years after application. Next to crop yield, several other characteristics were determined to obtain a better insight in how biochar influences the productivity of the aerobic rice cropping system. To verify the long lasting effect of wood biochar rate on soil chemical properties, such as soil pH and soil organic matter, annual measurements were done in the clay soil. To quantify the effect of biochar on soil water retention capacity, soil water retention curves for both the clay soil (in 1.5 and 2.5 years after biochar application) and the sandy soil (in 2 and 3 years after biochar application) were determined. To quantify the impact of wood biochar rate on N_2O-N fluxes, plot measurements were taken along four cropping seasons after biochar application in the clay Ferralsol.

This thesis consists of a general introduction (*Chapter 1*), four chapters addressing the research questions above (*Chapters 2 to 5*) and a general discussion (*Chapter 6*). *Chapter 2* focuses on the effects of wood biochar on rice yields and growth in 0.5 year after biochar application on the clay Ferralsol. Effects on yield components and soil chemical properties are discussed. *Chapter 3* focuses on the effect of biochar rate on soil water retention capacity of the sandy soil and the response of rice yield and yield components. We also present a new approach for modelling of soil water retention curves estimated from field experiments. It allows for formal statistical comparison of curves and adequate uncertainty assessments. Implications for the effect of biochar on soil water retention capacity and rice yields are discussed. *Chapter 4* focuses on the effect of biochar on soil chemical and physical properties and rice yields along three cropping seasons, up to 3.5 years after biochar application in the clay soil. The interaction of biochar with mineral N fertilization is discussed. The effect of biochar on soil water holding capacity and the remaining effects of biochar on soil chemical properties and its implications to aerobic rice production are discussed. *Chapter 5* focuses on the effect of biochar on N_2O-N fluxes along four cropping seasons on the clay soil, up to 2.5 years after biochar application. The overall effect of biochar on N_2O-N fluxes and soil related properties over the cropping seasons with special emphasis on the specific sub periods after N fertilization is discussed. *Chapter 6* provides the general discussion. It starts with a summary of the main findings of this study. Next, we reflect on the use of linear and nonlinear mixed models applied in this study and the novelty of its application in Agronomy. We discuss the importance of developing the statistical tools suitable for repeated measurements and spatially correlated data. We conclude with a discussion on the implications of using biochar for future farming in a tropical Savannah and suggest new directions for further research in this area.

Chapter 2

Biochar improves fertility of a clay soil in the Brazilian Savannah: short term effects and impact on rice yield

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"Nature is a language, can't you read?"
(Ask, The Smiths, 1986)

Abstract

The objective of this study was to report single season effects of wood biochar (char) application coupled with N fertilization on soil chemical properties, aerobic rice growth and grain yield in a clayey Rhodic Ferralsol in the Brazilian Savannah. Char application effected an increase in soil pH, K, Ca, Mg, CEC, Mn and nitrate while decreasing Al content and potential acidity of soils. No distinct effect of char application on grain yield of aerobic rice was observed. We believe that soil properties impacted by char application were inconsequential for rice yields because neither water, low pH, nor the availability of K or P were limiting factors for rice production. Rate of char above 16 Mg ha^{-1} reduced leaf area index and total shoot dry matter by 72 days after sowing. The number of panicles infected by rice blast decreased with increasing char rate. Increased dry matter beyond the remobilization capacity of the crop, and high number of panicles infected by rice blast were the likely cause of the lower grain yield observed when more than 60 kg N ha^{-1} was applied. The optimal rate of N was 46 kg ha^{-1} and resulted in a rice grain yield above 3 Mg ha^{-1}.

Keywords: aerobic system, carbonised biomass, Ferralsol, nitrogen, *Oryza sativa*, Oxisol

1. Introduction

Aerobic rice (*Oryza sativa*) based cropping systems (ARS) are typically rain fed, where rice is grown on well drained soils. Compared to rice paddy cultivation, less demand for labour, mechanization and effective water usage are important advantages of ARS (Pinheiro et al., 2006). However, grain yield of aerobic rice in the Brazilian Savannah (BS) can be 60% lower than in continuously waterlogged soil (Fageria, 2001). Crop yield constraints of ARS in the BS are: water limitation due to rainfall variability, high weed infestation, rice blast (*Magnaporthe grisea*), low soil organic matter (SOM) content and poor soil nutrient availability.

Approximately 46% of the total area in the BS is covered by Ferralsol (Embrapa, 2006), which have, in their original condition, favourable physical properties, such as fine texture, good structure and high water conductivity. Chemically, however, those soils are highly weathered, acid, with low SOM (Fageria et al., 2004). The SOM level is about 2% within 0.2 m soil depth. Under such soil conditions, nitrogen (N) is often the most yield-limiting nutrient. Management options that could increase N use efficiency would probably lead to increased aerobic rice yields in the BS.

One promising management option is the use of 'biochar' as soil amendment, although so far there are still no conclusive field studies that quantify the effect of biochar application on grain yield of aerobic rice in the BS. Pieces of charcoal produced from timber, worthless for industrial or domestic uses, could be recycled as soil amendment. It is such material that we refer to as 'biochar', a substance rich in resistant (pyrogenic) carbon (70–80% of the material) that might improve soil fertility. According to Jeffery et al. (2011), the main positive effects of biochar on crop yield are via increased soil water retention, reduced soil acidity and increased soil nutrient availability (in particular K). Using sprinkler irrigation and applying K and P fertilizer to all treatments, enabled us to focus on the potential interaction effect between biochar application and synthetic N fertilization on grain yield and yield components in ARS.

Some effects of biochar on ARS have been reported showing that the dominant effects are on soil chemical properties rather than on crop yield and that the positive effect of biochar on crop yield might be dependent on the application of organic or synthetic fertilizer (Haefele et al., 2011; Asai et al., 2009; Steiner et al., 2007). Further, Haefele et al. (2011) and Asai et al. (2009) reported some negative effects of biochar applications on aerobic rice growth and yield due to decreased N availability. To investigate the effect of wood biochar in combination with synthetic N on grain yield and on soil properties in ARS in areas of the BS, two field experiments were established: one on a clayey Rhodic Ferralsol featuring favourable soil conditions and with possibility for sprinkle irrigation and another on a less favourable sandy loam Dystric Plinthosol (non-irrigated). The short-term effects of wood biochar on ARS in the sandy loam Dystric Plinthosol were reported by Petter et al. (2012). The objective of our study was to report short-term effects of wood biochar application on soil chemical properties and on aerobic rice growth and grain yield on the clayey Rhodic Ferralsol in the BS.

2. Materials and methods

2.1. Experimental design and agronomic details

A field experiment was established in June 9, 2009, on a clayey Rhodic Ferralsol at the National Rice and Beans Research Centre, in Santo Antônio de Goiás, Goiás, Brazil (16°29’17”S and 49°17’57” W). The soil texture consisted of clay (574 g kg^{-1}), silt (100 g kg^{-1}), and sand (326 g kg^{-1}). Sixty four experimental plots of 40 m^2 (4 m× 10 m) were arranged in four replications, with wood biochar (0, 8, 16, 32 Mg ha^{-1}) and synthetic N (0, 30, 60, 90 kg ha^{-1}), each applied at four levels. At the establishment of the field trial, biochar was incorporated into 0–0.15m soil depth using a harrow. On November 3, 2009, following a crop of irrigated common beans (*Phaseolus vulgaris*), aerobic rice (*Oryza sativa*), cultivar ‘BRS Primavera’, was sown. Rice was sown in seven rows of 10m in each plot, with a row spacing of 0.4 m and a plant density of 100 seeds m^{-1}. Plots were located under a centre pivot. A total of 78 mm of water was applied by sprinkler irrigation in amounts of around 13 mm each time, throughout the growing season. Rates of 120 kg ha^{-1} of P and 60 kg ha^{-1} of K and 50% of the total N (urea) were applied to all plots and incorporated into the soil together with the rice seeds using a no-till planter. The remaining 50% of N was broadcasted at 33 days after sowing. Total rainfall during the growing season was 871 mm, average temperature was 24 °C, and average daily radiation was 18.5 MJ m^{-2}. Rice was harvested on 22nd of February, 2010.

2.2. Biochar properties

The source of biochar applied was charcoal produced from plantation timber (*Eucalyptus* sp.) by slow pyrolysis, at around 400–550 °C. Pieces of charcoal (≤ 8mm) were obtained from a commercial producer of charcoal in the region. It was milled to pass a 2 mm sieve before soil application. Chemical analysis showed that the biochar contained around 76% C, 0.8% N, 0.0002% P, 0.003% K, 0.0002% Cu+Fe+Mn+Zn, 0.05% Ca, and 0.009% Mg. This accounts for 77% of the biochar mass. The remaining mass of biochar comprises mainly O (ca. 20 %). Biochar has a labile fraction, around 6% of the total C, which was determined via oxidation of biochar with dichromate by the Walkley-Black method (Walkley & Black, 1934). Total C and N were determined in a PerkinElmer 2400 Series II CHNS/O Elemental Analyser. Extractable P, K, Cu, Fe, Mn, and Zn were determined using a Mehlich 1 solution (Mehlich, 1953); and available Ca and Mg were determined using a 1 mol L^{-1} KCl solution (Gavlak et al., 2003). Extraction was followed by determination via atomic absorption spectrometry (Embrapa, 2009).

2.3. Soil measurements

Soil chemical properties were determined using one 500 g composite sample for each plot collected in the 0–0.2m layer at 70 days after sowing (DAS). Available P, K, Cu, Fe, Mn, Zn, Ca and Mg were quantified using same methods as described previously. The pH was determined using a 1:1 soil:water solution (Bates, 1973); potential acidity (H + Al) using a solution of calcium acetate (0.5 mol L^{-1} at pH 7.1–7.2) followed by titration with NaOH (0.025 mol L^{-1}) using phenolphthalein (10 g L^{-1}) as indicator (Embrapa, 2009); and soil organic carbon (SOC) using the Walkley-Black method with sulphuric acid (H_2SO_4) to create internal heat for reaction (Nelson & Sommers, 1996). Cation exchange capacity (CEC) was calculated as the sum of Ca, Mg, K and Al.

Soil moisture, ammonium (NH^+_4) and nitrate (NO^-_3) concentrations were frequently measured throughout the growing season in the 0–0.1m soil layer; three subsamples were collected to get a 30 g soil sample in each plot. Around 10 g of soil was weighed before and after drying in an oven for 24 hours at 105 ºC. The available NH^+_4 and NO^-_3 were extracted by shaking 20 g of soil with 60 mL of 1 mol L^{-1} KCl solution for 60 minutes (Mulvaney, 1996). Extraction was followed by determination via flow injection analysis (Ocean Optics, USA). The final result was given in mg L^{-1}. To estimate mineral N [mg (kg soil)$^{-1}$] the soil moisture [(g water) (g soil)$^{-1}$] at the moment of sampling was taken into account.

2.4. Plant measurements

Total shoot dry matter (TDM) and leaf area index (LAI) were determined at 72 DAS; plants from a 0.5 m row (area of 0.2 m^2) were collected in each plot. Leaf area of 10 tillers was measured by using a LI-COR equipment (Lincoln, NE, USA). The total number of tillers in a 0.5 m row was counted and used to calculate LAI. All plants were dried in an oven at 75 °C for 48 hours and weighed to determine TDM. At 94 DAS, 20 panicles were collected to determine the number of panicles infected by rice blast (*Magnaporthe grisea*) in each plot. The number of infected panicles was visually determined. At 110 DAS, yield (weight of grains dried to 13% moisture) and yield components (total dry matter, number of tillers, panicles, grains, empty grains and full grains) were determined from an area of 2.4 m^2 (2 rows of 3 m). Spikelet fertility was expressed as the ratio between number of filled grains per panicle and total number of grains per panicle. Harvest index was calculated as the ratio between weight of dried grains and weight of TDM (including grains).

2.5. Statistical analysis

We adopted a generalized linear mixed model (GLMM) to estimate functional relationships (response surface) between soil and plant response variables and predictors (N and biochar). The GLMM allows the estimation of response surface parameters via restricted maximum likelihood method, accounting for spatial autocorrelation among plots via information on random effects. In our study, rows and columns (coordinates of plots) were included as random effects, and predictors were included as fixed effects. We started with a complete quadratic response surface model in which all predictors were included. To select the appropriate response surface model, we followed a backward criteria in which predictors with highest p-value ($p > 0.10$) were progressively excluded, subject to the hierarchical criteria of retaining the corresponding linear terms whenever interactions or quadratic terms were present (for further details see McCullagh & Nelder, 1983). Considering the expected high residual variance for most of the outcomes as consequence of the large experimental set, we adopted $p \leq 0.10$ as our threshold to safeguard against high type II error. Analyses were performed using the SAS/STAT® MIXED procedure (SAS Institute Inc., 2008).

3. Results

3.1. Soil chemical properties

Soil pH, Ca, Mg and CEC increased linearly with biochar rate, whereas for Al a linear decrease was observed (Table 1).

Table 1. Fitted response surface models to represent the quantitative effects of wood biochar (char) and synthetic nitrogen (N) rate on soil chemical properties of a clayey Rhodic Ferralsol cultivated with aerobic rice in the Brazilian Savannah, growing season 2009/2010 †.

Variables	Fitted models	R^2
P (mg kg^{-1})	32.64 -0.4390 N ** +0.005883 $N^{2\,***}$	0.49
K (mg kg^{-1})	59.37 +1.3705 char *** -0.1011 N ^{ns} -0.0127 char×N **	0.78
Cu (mg kg^{-1})	2.55 -0.01241 N ** +0.000118 $N^{2\,**}$	0.35
Mn (mg kg^{-1})	10.79 +0.1103 char *** -0.1052 N *** +0.000827 $N^{2\,**}$	0.79
Zn (mg kg^{-1})	4.94 -0.04723 N ** +0.000391 $N^{2\,*}$	0.51
Ca ($mmol_c$ kg^{-1})	10.09 +0.0879 char ** -0.04146 N *	0.60
Mg ($mmol_c$ kg^{-1})	3.26 +0.02292 char * -0.01213 N ^{ns}	0.61
pH (water)	5.06 +0.005716 char ** -0.00312 N **	0.70
H+Al ($mmol_c$ kg^{-1})	60.49 +0.31082 char ^{ns} +0.08002 N * -0.01299 $char^{2\,*}$	0.66
Al ($mmol_c$ kg^{-1})	3.41 -0.04703 char *** +0.01561 N *	0.67
SOC (g kg^{-1})	15.56 +0.00894 N *	0.15
CEC ($mmol_c$ kg^{-1})	18.47 +0.08376 char * -0.04542 N *	0.53
N-NH_4^+ (mg kg^{-1})	16.83 +0.06349 N ***	0.67
N-NO_3^- (mg kg^{-1})	43.85 +0.5002 char *** +0.3357 N ***	0.91

† Rate of char (0, 8, 16, 32 Mg ha^{-1}) and N (0, 30, 60, 90 kg ha^{-1}); SOC: soil organic carbon; CEC: capacity to exchange cations; R^2: squared Pearson correlation coefficient between observed and predicted values for each char×N treatment (n = 4). Nominal significance level of tests for effects: *** $p \leq 0.01$, ** $p \leq 0.05$, * $p \leq 0.10$, ^{ns} not significant.

The effect of biochar on potential acidity (H+Al) followed a quadratic trend with peak around 16 Mg ha^{-1}. The effect of biochar on K was dependent on the N application rate: the higher the rate of N, the more biochar was required to achieve same K concentration. There was no effect of biochar on both P and soil organic carbon (SOC) level. The SOC increased linearly with N application. A linear relationship was also observed between N rate and both nitrate (NO^-_3) and ammonium (NH^+_4) concentrations. Concentration of NO^-_3 increased linearly with biochar applications. Among micronutrients, Manganese (Mn) increased linearly with biochar application. There was no effect of biochar on Zn and Cu. Zn increased with N rate above 60 kg ha^{-1} and Cu above 53 kg ha^{-1}. There was no effect of N and biochar on Fe concentration.

3.2. Plant growth, grain yield and yield components

The influence of biochar on leaf area index (LAI) and total shoot dry matter (TDM) at 72 DAS followed quadratic trends with a peak around 16 Mg ha^{-1} (Table 2).

Table 2. Fitted response surface models to represent the quantitative effects of wood biochar (char) and synthetic nitrogen (N) rate on growth, grain yield and yield components of aerobic rice on a clayey Rhodic Ferralsol in the Brazilian Savannah, growing season 2009/2010 †.

Variables	Fitted models	R^2
LAI at 72 DAS (m^2 m^{-2})	3.83 +0.08097 char ** -0.00276 char$^{2\,**}$	0.27
TDM at 72 DAS (Mg ha^{-1})	3.67 +0.08908 char ^{ns} +0.02041 N ** -0.00329 char$^{2\,*}$	0.81
TDM at 110 DAS (Mg ha^{-1})	5.63 +0.05018 N * -0.00048 N$^{2\,*}$	0.53
Grain yield (Mg ha^{-1})	2.07 +0.03718 N ** -0.00036 N$^{2\,**}$	0.40
Harvest Index (Mg Mg^{-1})	0.44 -0.00221 char * -0.00072 N ^{ns} +0.00004 char×N **	0.28
Weight of 1000 grains (g)	21.91 -0.01586 N **	0.36
Spikelet Fertility × 100 (%)	0.72 +0.00201 N * -0.00003 N$^{2\,**}$	0.52
Healthy Panicles × 100 (%)	0.59 +0.00348 char * -0.0016 N *	0.45

† Rate of char (0, 8, 16, 32 Mg ha^{-1}) and N (0, 30, 60, 90 kg ha^{-1}); R^2: squared Pearson correlation coefficient between observed and predicted values for each char×N treatment (n = 4). DAS: days after sowing. Nominal significance level of tests for effects: ** $p \leq 0.05$, * $p \leq 0.10$, ^{ns} not significant.

Predicted LAI (m^2 m^{-2}) varied from 4.4 ± 0.49 (16 char) to 3.6 ± 0.51 (32 char), regardless of N. Predicted TDM (Mg ha^{-1}) varied from 6.1 ± 0.54 (16 char; 90 N) to 3.1 ± 0.61 (32 char; 0 N). There was no effect of biochar on grain yield of aerobic rice. Rates of N above 60 kg ha^{-1} tended to negatively affect grain yield, even though the higher the rate of N, the higher the TDM at 72 DAS. Predicted grain yield (Mg ha^{-1}) varied from 2.99 ± 0.32 (60 N) to 2.07 ± 0.38 (0 N) (Fig.1). As observed for grain yield, the influence of N on TDM at 110 DAS and on spikelet fertility (SF) also followed a quadratic trend, with predicted TDM (Mg ha^{-1}) at 110 DAS varying from 6.9 ± 0.55 (60 N) to 5.6 ± 0.63 (0 N). Predicted SF varied from 0.75 ± 0.02 (30 N) to 0.65 ±0.02 (90 N). Weight of 1000-grains decreased linearly with N rate. Predicted weight of 1000-grains varied from 21.91 ± 0.42 (0 N) to 20.48 ± 0.45 (90 N). There was, however, an effect of the interaction biochar×N on harvest index (HI), whereby the higher the rate of biochar applied, the more N was required to achieve the same HI. In treatments without N application, biochar rate had a negative effect on HI; and in treatments without biochar application, N rate had a negative effect on HI. Predicted HI (Mg Mg^{-1}) varied from 0.44 ± 0.03 (0 char; 0 N) to 0.37 ± 0.03 (32 char; 0 N). Ratio of healthy panicles (HP) increased linearly with biochar rate, whereas N rate linearly decreased HP ratio (Fig.1). Predicted HP varied from 0.70 ± 0.07 (32 char; 0 N) to 0.45 ± 0.06 (0 char; 90 N).

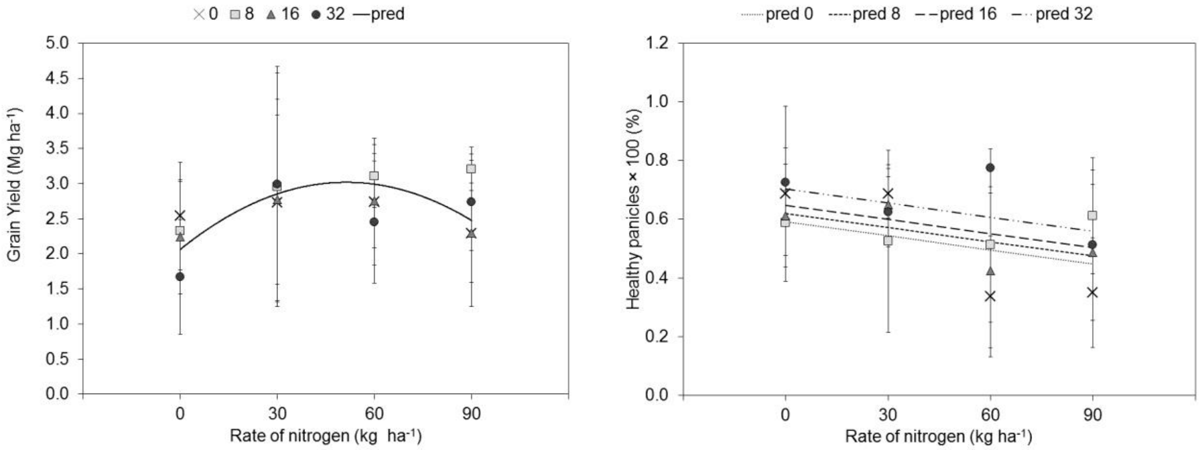

Figure 1. Response surfaces for the effect of wood biochar (×0, □8, Δ16 and ○32 Mg ha^{-1}) and synthetic nitrogen (0, 30, 60 and 90 kg ha^{-1}) rate on grain yield and percentage of healthy panicles of aerobic rice cultivated on a clayey Rhodic Ferralsol during 2009/2010 growing season in the Brazilian Savannah. Dots represent observed values for each char×N treatment (n = 4). Lines correspond to predicted values (pred) of the fitted models shown in Table 2.

4. Discussion

We found no observable effect of biochar on grain yield of aerobic rice, even though biochar application led to improvements on soil chemical properties, mostly related to the acidity neutralizing capacity of biochar. We postulate that the observed improvements in soil properties due to biochar application did not increase grain yield because (i) there was no limitation of water, K or P in our study and (ii) aerobic rice is an acid tolerant crop (Fageria et al., 2004). For this study we expected N to be the most important yield-limiting factor. Soil nitrate concentration increased with biochar applications without influencing plant growth, except at very high levels of biochar (> 16 Mg ha^{-1}) that reduced TDM and LAI at 72 DAS. Similarly, Asai et al. (2009) observed that application of 16 Mg ha^{-1} wood biochar reduced chlorophyll concentration in rice leaves. The cause for a negative effect on plant growth was not clear in our study. Possibilities are discussed below.

Throughout the growing season, nitrate was the predominant form of mineral N in the soil with the presence of biochar. If there was an excess of nitrate in soil, then sporadic losses via denitrification were prone to occur, especially under irrigated systems (Bouwman, 1990). Complementary, due to the high C/N ratio of the wood biochar and its labile C (ca. 6% of the total C), soil microbial activity is likely to raise leading to a decrease in N availability to the crop. In an Amazon Ferralsol amended with wood biochar, Lehmann et al. (2003) reported a decrease in total soil N availability and Steiner et al. (2008) detected an increase in microbial activity with increasing wood biochar rate. If there was an effect of biochar on decreasing N availability to the crop, then it was temporary, since no effect of biochar on TDM and yield at crop maturity was detected in this study. Conversely, surplus fertilization with synthetic N increased TDM at 72 DAS but lowered yields because it could result in an amount of dry matter that exceeded the capacity of the rice variety to remobilize stored carbohydrates to fill grains. This capacity limitation is more usually found in modern varieties, such as the 'BRS Primavera' used in this study, than in traditional aerobic rice varieties (Pinheiro et al., 2006).

For our study and according to our model, the optimal rate of N was around 46 kg ha^{-1} resulting in a grain yield above 3 Mg ha^{-1} (Fig. 1). In a less favorable area (76% sand, non-irrigated) in the Brazilian Savannah, Petter et al. (2012) found that grain yield of aerobic rice was increased by 3% per Mg ha^{-1} biochar amendment, with the rice sown one month after wood biochar application. This finding suggests that short-term effects of biochar application on grain yield of aerobic rice can be more prominent under less favorable (Petter et al., 2012) than favorable (this study) conditions in the Brazilian Savannah. Clay soils naturally have higher water holding capacity and CEC than sandy soils. In sandy soils, wood biochar is likely to act as an extra fertilizer, as well as increasing water holding capacity as reported by Tryon (1948).

Apart from the positive effects of biochar on improving soil fertility, we observed that biochar applications decreased the number of panicles infected by rice blast (*Magnaporthe grisea*), whereas N rate increased the severity of the disease. While leaf blast can indirectly reduce grain yield via reduction of green leaf area (Bastiaans, 1993), rice blast in panicles directly affects the number and weight of grains (Silva-Lobo et al., 2012) as was observed in this study. The effect of biochar on increasing the number of healthy panicles might be associated with Manganese (Mn). Manganese plays an important role for the resistance to rice blast (Thompson& Huber, 2007; Primavesi et al., 1972). We observed that biochar application increased soil Mn concentration, but we did not find an observable direct effect of soil Mn on healthy panicles, spikelet fertility or harvest index (data not shown). This suggests that the effect of biochar on reducing rice blast in panicles has probably other causes than Mn alone.

Elad et al. (2010), for example, has reported a systemic resistance to foliar fungal pathogens induced by soil-applied wood biochar. Since rice blast is one of the most yield-limiting factors of aerobic rice in Brazil, the potential positive effect of biochar on resistance to rice blast requires further investigation. The negative effect of rate of N > 60 kg N ha^{-1} on grain yield can have two causes: increased dry matter beyond the remobilization capacity of the crop; and increased number of panicles infected by rice blast. Effects of biochar on soil properties may change with time, leading to different responses than the ones observed in this study.

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Chapter 3

Biochar increases plant-available water in a sandy loam soil under an aerobic rice crop system

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"Não basta abrir a janela para ver os campos e o rio.
Não é bastante não ser cego para ver as árvores e as flores."
(Alberto Caeiro, heterônimo de Fernando Pessoa, 1925)

Abstract

The main objective of this study was to assess the impact of biochar rate (0, 8, 16 and 32 Mg ha^{-1}) on the water retention capacity (WRC) of a sandy loam Dystric Plinthosol. The applied biochar was a by-product of slow pyrolysis (~ 450 ºC) of eucalyptus wood, milled to pass through a 2000 μm sieve that resulted in a material with an intrinsic porosity ⩽ 10 μm and a specific surface area of ~ 3.2 m^2 g^{-1}. The biochar was incorporated into the top 15 cm of the soil under an aerobic rice system. Our study focused on both the effects on WRC and rice yields 2 and 3 years after its application. Undisturbed soil samples were collected from 16 plots in two soil layers (5-10 and 15-20 cm). Soil water retention curves were modelled using a nonlinear mixed model which appropriately accounts for uncertainties inherent of spatial variability and repeated measurements taken within a specific soil sample. We found an increase in plant available water in the upper soil layer proportional to the rate of biochar, with about 0.8% for each Mg ha^{-1} biochar amendment at 2 and 3 years after application. The impact of biochar on soil WRC was most likely related to an effect in overall porosity of the sandy loam soil, which was evident from an increase in saturated soil moisture and macro porosity with 0.5% and 1.6% for each Mg ha^{-1} of biochar applied, respectively. The increment in soil WRC did not translate into an increase in rice yield, essentially because in both seasons the amount of rainfall during critical period for rice production exceeded 650 mm. The use of biochar as a soil amendment can be a worthy strategy to guarantee yield stability under short-term water-limited conditions. Our findings raise the importance of assessing the feasibility of very high application rates of biochar and the inclusion of a detailed analysis of its physical and chemical properties as part of future investigations.

Keywords: biochar, sandy loam, water retention capacity, nonlinear mixed model, aerobic rice

1. Introduction

Soil water retention capacity (WRC) is a potential indicator of soil quality and productivity. Several agronomic practices such as no-tillage, mulching and cover crops are implemented aiming to improve soil physical properties. An enhanced soil WRC through the adoption of these practices is attained via protection of the soil surface, improved soil aeration and infiltration, or an increased soil organic matter level. Of particular relevance for protection of soil surface, the use of mulching is regarded as an effective option (Fernández et al., 2012; Lee et al., 2013; Prats et al., 2013). However, according to Mcdonagh et al. (2014), improved soil management practices likely to be adopted by land users are multi-purpose technologies. In this context, the use of carbonised biomass, or biochar, has been regarded as an interesting option for improving soil physical properties (Glaser et al., 2002).

The rising demand for charcoal by iron smelters in Brazil has resulted in a rapid increase in the area covered with timber plantations. Between 2005 and 2010 the total increase was 23%. In 2010, forest plantations in Brazil covered six million hectares of which 73% comprised of eucalyptus forests. In comparison to natural vegetation, land use with eucalyptus plantation might not have a negative impact on soil organic carbon content (Fialho and Zinn, 2012). To the contrary, the cutting of native vegetation for charcoal production can result in highly degraded land, due to a drastic decrease in soil organic matter content and increase in soil bulk density (Araújo et al., 2013). Of all produced wood in Brazil, around 35% was destined to charcoal production (ABRAF, 2010). Small pieces of char (< 8 mm) have to be compacted into bricks if they are to be used as charcoal by iron smelters. Alternatively, these pieces can be recycled as soil amendment. Potentially, a large quantity of this type of biochar is available for Brazilian farmers. It is this material that was tested in the current study.

Tryon (1948) showed that available soil moisture in a sandy soil increased linearly with increasing wood biochar application rate. Several recent studies have also reported the potential of wood biochar to increase WRC of sandy soils (Pereira et al., 2012; Dempster et al., 2012; Basso et al., 2013; Abel et al., 2013; Ibrahim et al., 2013). The majority of studies were conducted under artificially controlled conditions, testing the effect of a wide range of biochar amounts on WRC. Though such studies are useful, the extrapolation of their results to field conditions presents some limitations: i) the amounts of biochar tested are often larger than what is practically and economically feasible for incorporation into agricultural fields; ii) the conditions for biochar application in artificially packed soil samples might lead to artefacts not normally encountered under field conditions, where biochar is incorporated via tillage and crops are grown afterwards; and iii) the consolidation time is usually shorter in artificially controlled conditions than under field trials. Thus, more long term studies on the effect of biochar under field conditions are required.

The increment in available water following biochar application is commonly related to the porous structure of the material. The pores behave as additional capillaries, favouring the WRC of the soil. Primarily, the number and size of pores is determined by the type of feedstock, temperature level and time of pyrolysis. The specific surface area (SSA) of biochar increases with temperature of pyrolysis (Lei and Zhang, 2013; Bornemann et al., 2007). At temperatures of 450 ºC the SSA can be smaller than 10 m^2 g^{-1}, while at temperatures of 600-750 ºC it can rise to around 400 m^2 g^{-1} (Kookana et al., 2011). Clearly, SSA is a characteristic that should be considered when the impact of biochar on soil WRC is investigated. Secondly, the particle size of biochar can be a determinant of the potential positive effect on soil WRC. Tryon (1948) showed that the impact on soil WRC was higher with finer material (< 1000 μm) than with larger particle sized biochar (2000-5000 μm).

The soil WRC is represented by the nonlinear relation between volumetric soil moisture and matric potential, referred to as the soil water retention curve (SWRC). Such curves can be used as indicators of changes in soil physical properties caused by the incorporation of biochar into the soil matrix. The van Genuchten model (van Genuchten, 1980) is one of the most widely used representations of the soil WRC. Generally, statistical programs specifically designed to fit SWRC only allow fitting of curves for isolated treatments, without accounting for experimental structure (e. g., Dourado-Neto et al., 2000). The isolated treatment-specific model fitting has three main disadvantages: i) comparison of SWRC between treatments via formal statistical tests is not possible due to the absence of an error structure that accounts for overall variance within treatments; ii) autocorrelations among random errors of moisture measurements taken in the same sample unit (the cylinder) under different matric potentials are ignored, leading to incorrect quantification of model uncertainty; and iii) the spatial variability, likely to be high under field conditions, cannot be fully accounted for (Omuto et al., 2006). In this study we propose the use of nonlinear mixed (NLM) model to overcome these disadvantages.

Circa 40% of overall Brazilian crop production is located in the Brazilian Midwest region (IBGE, 2012), where our study was conducted. The predominant biome in this region is a tropical savannah. Though a tropical savannah is a drought prone environment (Peel et al., 2007), Brazilian farmers usually manage to grow two crops during the wet season (from October to March). However, rising temperatures and changes in rainfall distribution pattern have decreased the chances of an economically successful second harvest. Further temperature rises are projected to provoke decreases in suitable area for cultivation of the majority of crops in Brazil, mainly due to an increase in evapotranspiration (Assad et al., 2008). This further stresses the need of agronomic measures able to increase the water use efficiency in crop production.

The current study is a continuation of the experiment described by Petter et al. (2012), in which they showed that rice yields increased with around 3% per Mg ha^{-1} biochar amendment in the first and second seasons after application. Additionally, in a pot experiment using a sterile sand, Pereira et al. (2012) observed an increase in soil WRC at matric potentials lower than -6 kPa with a rate of 12% w/w of a similar biochar as the one tested in this study, accompanied by a delay in the point where rice transpiration rate is affected by water stress and declines. Hence, the main objective of this study was to test the impact of a range of wood biochar rates (up to 1.5% w/w) on both soil WRC and rice yields on a sandy loam Dystric Plinthosol at 2 and 3 years after application under field conditions. As part of this endeavour, we introduce the use of a nonlinear mixed (NLM) model for estimating shape parameters of the SWRCs.

2. Material and Methods

2.1. Experimental set up and biochar characterization

In 2008, a permanent non irrigated field trial was set up at Estrela do Sul Farm in Nova Xavantina, Mato Grosso, in the Brazilian Midwest region (14°34 '50"S and 52º 24' 01"W) on sandy loam Dystric Plinthosol (76% sand, 17% clay). The Köpper-Geiger climate classification of the region is Aw (Peel et al. 2007). The monthly precipitation and average temperatures since the start of the field trial are presented in Fig. 1, based on data from Agritempo (2014). Details on the history of the field trial and soil chemical properties can be found in Petter et al. (2012), who reported on the influence of biochar application on rice growth and yields at one month and at one year after application. Here we report on the most recent growing seasons of rice: from 13 December 2010 to 2 April 2011, and from 13

December 2011 to 2 April 2012, corresponding to two (S2) and three (S3) years after biochar application, respectively. Our analysis focuses on the influence of biochar on two variables, namely soil WRC and rice yields.

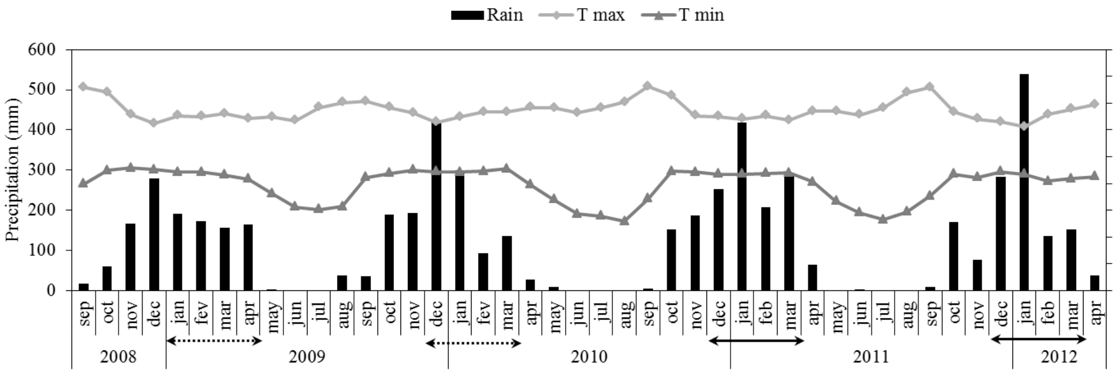

Figure 1. Monthly precipitation (Rain) and average of maxima (T max) and minima (T min) temperatures since application of biochar in the field trial in Nova Xavantina, MT, Brazil. Solid arrows indicate rice-growing seasons S2 and S3. Dotted arrows represent previous seasons reported by Petter et al. (2012).

Biochar was applied once, when the field trial was established on 5 December, 2008. Four levels of mineral fertilisation were applied in strips, and the four levels of biochar (0, 8, 16 and 32 Mg ha^{-1}) where applied within the strips in a randomized block design, with four replicates. Sixteen treatments were used, resulting in a total of 64 experimental plots, each with an area of 40 m^2 (4 × 10 m). Mineral fertilisation was always applied in strips across the four blocks. In S2 and S3, four levels of N-fertilisation (0, 30, 60 and 90 kg N ha^{-1}) were applied and all plots were given the same rate of P-K (kg ha^{-1}) at sowing (60-20 in S2, and 30-30 in S3) taking into account the soil chemical analysis prior to sowing and fertiliser recommendations for aerobic rice systems in the Brazilian savannah (EMBRAPA, 2007). The N-fertiliser (urea) was divided into three applications: at sowing and at 25 and 45 days after emergence (DAE). Rice (BRS Primavera) was sown directly with a 5-row Semeato® planter adapted for no-tillage systems, with space between rows of 45 cm and 110 seeds m^{-1}. Weeds infestation was chemically controlled with Glyphosate® (5 L ha^{-1}) applied at around 15 days prior to sowing and with 2-4 D (0.7 L ha^{-1}) or Star Rice® (0.4 L ha^{-1}) around 10 DAE. Additionally, manual weeding operations were conducted at around 45 and 75 DAE.

Air dried biochar (particle size ≤ 2000 μm) was spread manually on the soil surface, and incorporated into the upper 15 cm, using a harrow. The amount of biochar applied to the upper 15 cm was based on the average amount of pyrogenic C found in the fertile anthropogenic dark earths (ADE) of the Amazon. According to Glaser et al. (2001) the upper 30 cm of the ADE soils contain around 25 Mg ha^{-1} pyrogenic C, corresponding to an amount of 12.5 Mg ha^{-1} within 0-15 cm soil layer. As the biochar tested in our field trial had a concentration of 77% pyrogenic C, we applied a lower (8 Mg ha^{-1}), similar (16 Mg ha^{-1}) and higher (32 Mg ha^{-1}) rate of biochar than the amount of pyrogenic C found in ADE. Considering the soil bulk density and depth where biochar was applied, the application rate on a dry mass basis (weight of biochar / total weight of soil), was equivalent to 0.4, 0.7, and 1.5% w/w. The biochar was made of eucalyptus timber via slow pyrolysis in a cylindrical metal kiln using temperatures around 400-500 °C. A single point surface area of biochar was determined by the Brunauer, Emmelet and Teller (BET) nitrogen absorption method (Brunauer et al., 1938), using nitrogen gas sorption analysis at 77.3 K (-195.9 ºC).

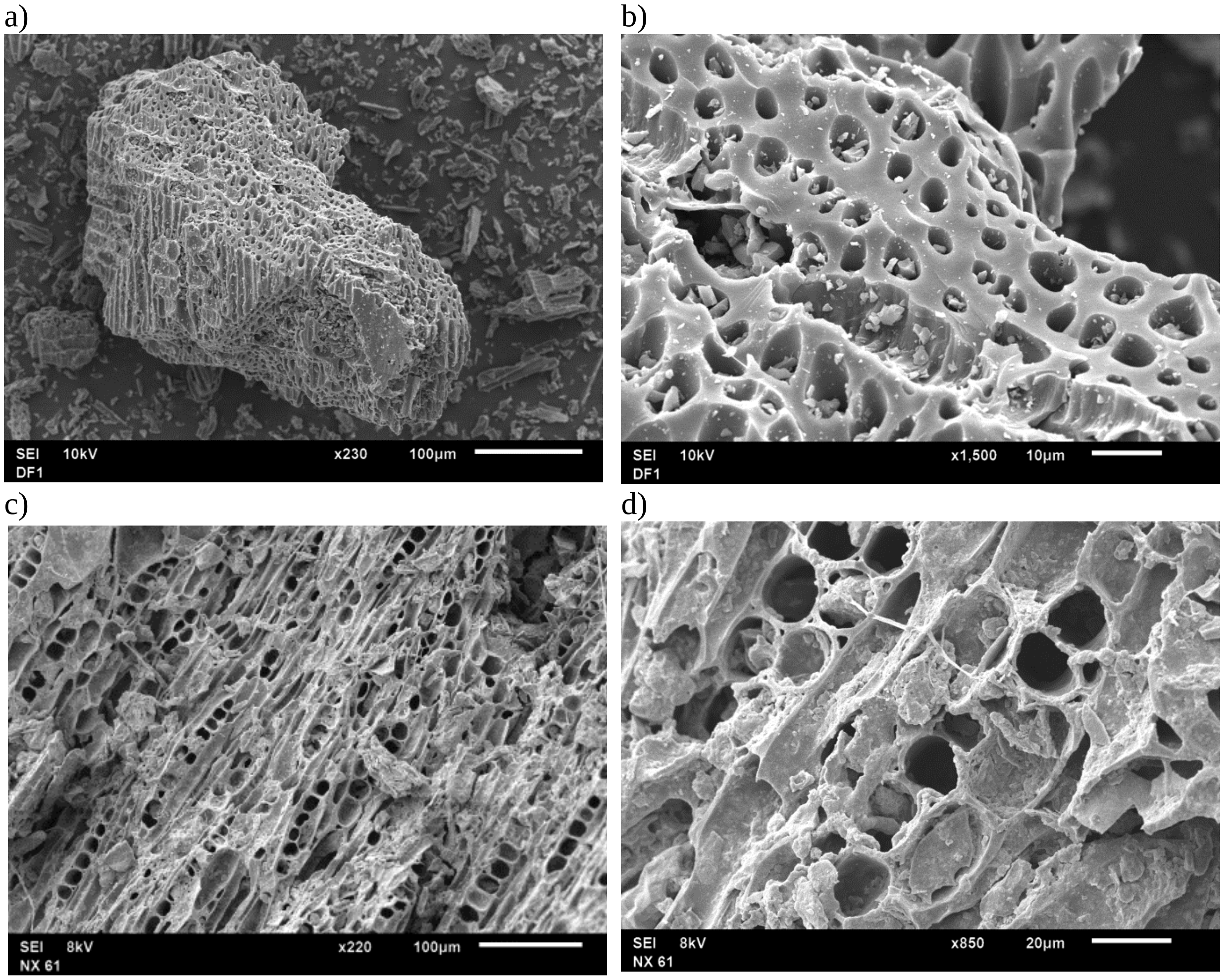

Figure 2. High-resolution images of Eucalyptus wood biochar (particle size ≤ 2000 µm) before application (a, b) and 2 years after application into a sandy Dystric Plinthosol (c, d). Images made at LABMIC, Institute of Physics, Federal University of Goiás.

The specific surface area (SSA) of the biochar applied, with a bulk density of 0.3 g cm^{-3}, was 3.2 ± 0.5 m^2 g^{-1}. The porous structure of the biochar (pore size ≤ 10 μm) is shown in Fig. 2.

The high resolution images were made using a Scanning Electron Microscope (SEM), Jeol, JSM – 6610, equipped with EDS, Thermo scientific NSS Spectral Imaging. The samples were covered with a gold film before analysis with the equipment Denton Vacuum, Desk V. Chemical properties of biochar were described in Petter et al. (2012).

2.2. Measurements on soil WRC and the modelling of SWRCs

The soil WRC was evaluated at two (S2) and three (S3) years after biochar application. Soil samples (cylinders of inox steel of 5 cm height and 5 cm diameter) were collected from mini-trenches 50 cm deep between rows of rice around 75 DAE. Setting of mini-trenches was completely randomized among two strips located at the right and left borders of the field trial (2 replicates for each biochar rate within each strip). Since the biochar was incorporated into the upper 15 cm layer, soil samples were collected in the centre (5-10 cm) and just below (15-20 cm) this layer to account for an effect of biochar that had possibly moved out of the original layer. Samples were collected from 16 plots (4 biochar rates x 4 plots, one sample per soil layer per plot) in a moist soil on 15 March, 2011 and on 3 March, 2012. The soil WRC was determined according to EMBRAPA (1997) adapted from Freitas Jr. and Silva (1984). Samples were saturated with water for 12 h and analysed in a centrifuge Kokusan H-1400pF®, four samples at a time, for 30 minutes under seven speed levels: 600, 700, 800, 1300, 1800, 2400 and 9100 rpm (equivalent to 0, 33.00, 44.92, 58.67, 154.93, 297.03 and 528.05 *g*). The volume of the soil water in the samples subjected to different speeds corresponded to seven matric potentials: -6, -8, -10, -33, -60, -100 and -1500 kPa. The bulk density was determined as the ratio between the dried mass of soil and the volume of a cylinder. The bulk density was used to calculate the volumetric soil moisture (cm^3 cm^{-3}). Saturated soil moisture was determined as the soil moisture content in saturated samples at 0 kPa right before subjecting samples to different speeds in the centrifuge.

The relation between observed volumetric soil moisture and soil matric potential (the soil water retention curve - SWRC) was determined by fitting the van Genuchten model described in Eq. (1).

$$\theta(\psi) = \theta_r + (\theta_s - \theta_r)\left[\frac{1}{1+(\alpha\psi)^n}\right]^m, \tag{1}$$

where $\theta(\Psi)$ is the volumetric soil moisture ($cm^3\ cm^{-3}$) at a given matric potential Ψ (kPa); θ_r is the residual soil moisture (soil moisture content at a $\Psi \geq$ -1500 kPa); θ_s is the saturated soil moisture (soil moisture content at 0 kPa); and m, α, and n are shape parameters. The Mualen constraint m = 1-1/n (Mualem, 1976) was adopted to increase model parsimony.

We used a nonlinear mixed (NLM) model for uncertainty assessment of SWRC estimates by considering the whole experimental design structure to quantify residual variance. For parsimony and to reduce the risk of non-convergence, we set θ_r and θ_s as known parameters. By adopting such an approach, the quantification of uncertainty of shape parameters α and n and the test of the null hypothesis of interest were performed considering the overall variance of soil moisture arising from within treatments variance. Further, the NLM model permits accounting for potential random effects associated to plot location, as proposed by Omuto et al. (2006). In our study, correlations among measurements taken within the same sample unit (one cylinder per plot for each soil depth) were accounted for by including plot as a random effect u in the model. The core of the NLM model adopted is the van Genuchten-Mualen model (Eq. (1)). The generic NLM model used to estimate the SWRC for each biochar level within two soil layers and two years was given by Eq. (2):

$$Y_{ijk} = \bar{\theta}_{r(i)} + \left(\bar{\theta}_{s(i)} - \bar{\theta}_{r(i)}\right)\left[\frac{1}{1+(\alpha_i \psi_{ijk})^{n_i}}\right]^{1-1/n_i} + u_{ij} + e_{ijk}, \tag{2}$$

where Y_{ijk} is the observed soil moisture of the treatment level i (i = 0, 8, 16, 32 Mg ha^{-1}) in the replication j (j = 1, 2, 3, 4) at a matric potential k (k = -6, -8, -10, -33, -60, -100 kPa); $\bar{\theta}_{r(i)}$ is the residual soil moisture in the treatment level i, averaged over observed values $\theta_{r(ij)}$ in j replicates; and $\bar{\theta}_{s(i)}$ is the saturated soil moisture in the treatment level i averaged over observed values $\theta_{s(ij)}$ in j replicates; α_i and n_i are the shape parameters for each treatment level i; $u_{ij} \sim$ N (0, Σ) represents the random effect of latent variables associated with location of a plot ij (ij = 1, …, 16); and $e_{ijk} \sim$ N (0, σ^2) is the random error associated with each measurement Y_{ijk}.

The residual soil moisture ($\theta_{r(ij)}$) was assumed as the measured soil moisture content at -1500 kPa and the saturated soil moisture ($\theta_{s(ij)}$) as the measured soil moisture content at 0 kPa. Shape parameters were estimated using the maximum likelihood method, implemented in NLMIXED Procedure of the SAS/STAT® software (SAS Institute Inc., 2008). Comparisons of shape parameters between control and treatments with biochar were performed by *t*-tests for linear contrasts.

2.3. Analysis of soil physical-hydric variables response to biochar rate

The response of some key soil physical-hydric variables to biochar rate was evaluated via measurements of: i) soil bulk density (BD); ii) saturated soil moisture (θ_s); iii) residual soil moisture (θ_r); iv) macro porosity (MAC), as the predicted soil moisture content between 0 and -6 kPa ($\hat{\theta}_0 - \hat{\theta}_6$); v) rice available water (RAW), as the predicted soil moisture content between -6 and -100 kPa ($\hat{\theta}_6 - \hat{\theta}_{100}$); and vi) plant available water (PAW) as the predicted soil moisture content between -6 and -1500 kPa ($\hat{\theta}_6 - \hat{\theta}_{1500}$). The predicted volumetric soil moisture ($\hat{\theta}$) was estimated via the model described in Eq. (2). The RAW was also estimated considering that the critical soil water volume for rice production should be defined at a matric potential of -100 kPa according to Wopereis et al. (1996).

Response of physical-hydric soil variables to biochar rate were analysed for each year and soil layer separately via the quadratic model described in Eq. (3):

$$y_{ij} = \beta_0 + \beta_1 char_i + \beta_2 char_i^2 + e_{ij}, \qquad (3)$$

where y_{ij} is the observation of the response variable y corresponding to biochar level i (i = 0, 8, 16, 32 Mg ha^{-1}) of the replication j (j = 1, 2, 3, 4); β_0 is the intercept; β_1 and β_2 are the linear and quadratic effects of biochar, respectively; and $e_{ij} \sim N(0, \sigma^2)$ is the random error associated to each observation y_{ij}.

Analyses were performed using the MIXED procedure (Proc MIXED) of the statistical software SAS/STAT® (SAS Institute Inc., 2008). The magnitude of the biochar effect was assessed by nominal significance levels (p-values) derived from hypothesis testing of β_1 and β_2 estimates. Due to the large experimental area, relatively high residual variances were anticipated to occur. For that reason, we adopted 0.10 as the appropriate p-value for the selection of model predictors in order to safeguard against high type II error.

2.4. Measurement and analysis of rice yield and yield components

The response of rice yield and yield components was measured for all biochar and N-fertilisation treatments. At crop maturity, around 100 DAE, total shoot dry matter, grain yield (weight of rice grains dried to 13% moisture) and yield components (number of panicles, grains panicle^{-1}, grain filling index and 1000-grain weight) were determined in samples collected from 2 rows of 3 m in the centre of each plot. Harvest index was calculated as the ratio between grain yield and total shoot dry matter. Filled and unfilled grains from panicles within the harvested area were separated with a vertical blower and counted with a seed counter. Grain filling index was calculated as the ratio between the number of filled grains and the total number of grains.

We used a linear mixed model instead of the commonly used design based ANOVA to analyse the data due the incomplete randomisation of N treatments. The linear mixed model adopted allowed us to account for potential spatial auto-correlation among plot measurements. Location of a plot was established by its position in a specific block and row within a block. The location of a plot was included as a random effect. Biochar, N, biochar×N and quadratic terms were included as fixed effects. Model parameters were estimated by the restricted maximum likelihood method – REML. Analyses were performed using the Mixed Procedure (Proc MIXED) of the statistical software SAS/STAT® (SAS Institute Inc., 2008). Graphical residual analysis, influence diagnostics and checking for potential violation of model assumption were conducted using the ODS GRAPHICS option. Response surfaces for identifying patterns of response of rice yields and yield components to biochar and N treatments were modelled for each season separately. A complete quadratic model (Eq. (4)) in which all predictors (biochar, N and biochar×N) were included was the starting point:

$$y_{ijbr} = \beta_0 + \beta_1 char_i + \beta_2 N_i + \beta_3 char_i * N_i + \beta_4 char_i^2 + \beta_5 N_i^2 + c_b + d_r + e_{ijbr}, \tag{4}$$

where y_{ijbr} is the observation of the response variable y corresponding to biochar and N treatments i (i = 1, 2, 3, 4, …, 16) of the replication j (j = 1, 2, 3, 4); β_0 is the intercept; β_1 and β_2 are linear effects of biochar and N, respectively; β_3 is the interaction effect biochar×N; β_4

and β_5 are quadratic effects of biochar and N, respectively; c_b and $d_r \sim$ N (0, Σ) are the potential random effects related to location of a plot in a block *b* (b = 1, 2, 3, 4) and in a row *r* (r = 1, 2, 3, 4) within a block *b*; and $e_{ijbr} \sim$ N (0, σ^2), the random error associated to each observation y_{ijbr}.

Again, we adopted 0.10 as the appropriate p-level in the process of predictors' selection. To determine the appropriate response surface, predictors containing the highest p-value (p > 0.10) were progressively excluded respecting the hierarchy of effects: linear terms were retained whenever interaction or quadratic terms were significant (MacCullagh and Nelder, 1983). The magnitude and evidence of the effects was assessed by estimates and their respective nominal significance levels.

3. Results

3.1. The use of NLM to adjust SWRCs and effects of biochar rate on shape parameters

Overall, the goodness of fit was high (R^2: 0.77 to 0.98), indicating the adequacy of the proposed nonlinear mixed (NLM) model to estimate the shape parameters of the SWRCs (Table 1). Inclusion of the random effect *u* significantly increased the accuracy of the SWRC

Table 1. Estimates of shape parameters of the Van Genuchten model fitted to represent soil water retention within 5-10 cm and 15-20 cm layers at two (S2) and three (S3) years after application of 8, 16 and 32 Mg ha^{-1} biochar into a sandy Dystric Plinthosol.

Treatment	Parameter estimates (5-10 cm)		R^2	Parameter estimates (15-20 cm)		R^2
	-----------α-------	---------n---------		-----------α---------	---------n-------	
	S2					
control	0.1110 (0.0533)	1.578 (0.093)	0.94	0.0344 (0.0147)	1.656 (0.088)	0.94
8	**0.0154* (0.0052)**	**1.882* (0.110)**	0.83	**0.0061* (0.0023)**	**1.951** (0.103)**	0.83
16	0.1443 (0.0725)	1.533 (0.088)	0.95	0.0760 (0.0371)	1.513 (0.075)	0.89
32	**0.0166* (0.0056)**	1.794 (0.089)	0.77	0.0131 (0.0055)	1.741 (0.087)	0.82
	S3					
control	0.0651 (0.0168)	1.677 (0.071)	0.97	0.0661 (0.0175)	1.653 (0.065)	0.97
8	0.0723 (0.0150)	1.738 (0.081)	0.95	0.0895 (0.0196)	1.678 (0.067)	0.97
16	0.0969 (0.0204)	1.707 (0.074)	0.98	0.1049 (0.0253)	1.675 (0.075)	0.96
32	0.0622 (0.0110)	1.781 (0.078)	0.97	0.0410 (0.0113)	1.636 (0.052)	0.94

Standard error of estimates are within brackets (n = 4). Nominal significance level of *t*-test for contrasts between control and treatments with biochar within season and soil layer: ** $p \leq 0.05$ and * $0.05 < p \leq 0.10$; R^2: the squared Pearson correlation coefficient between measured and predicted soil moisture means (n = 24).

modelling (Fig. 3). The consistent SWRC underestimation at high matric potential was likely due to increases in soil moisture content with biochar application, which was particularly evident from SWRCs for treatments with 8 and 32 Mg ha^{-1} in the upper and lower soil layers in S2.

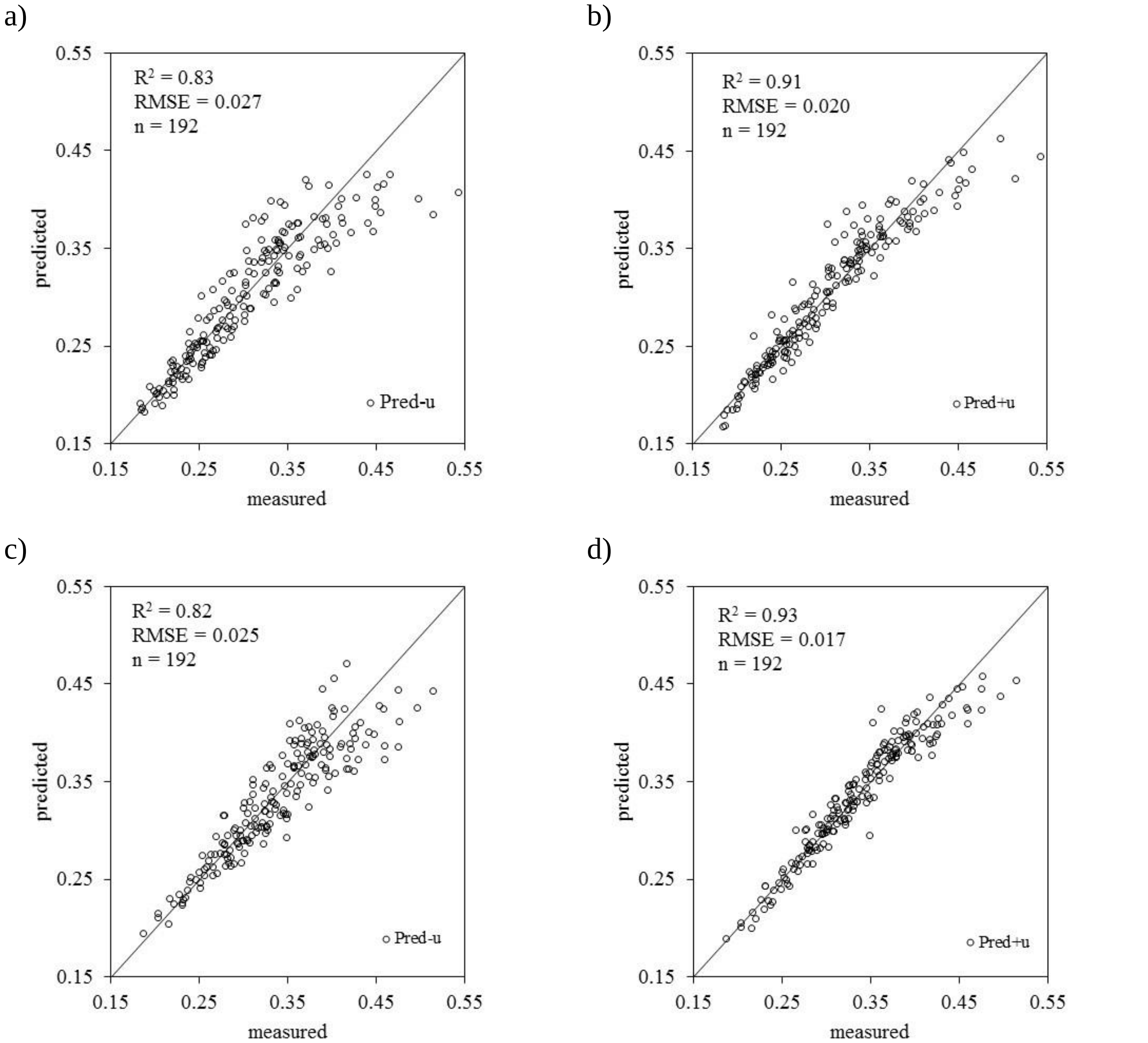

Figure 3. Goodness of fit of the nonlinear mixed model used to predict soil water retention capacity, summarized via correlation coefficient (R^2) and root of mean square error (RMSE). Agreement between measured and predicted moisture values (a, c); agreement between measured and predicted moisture values including the random effect u in the model (b, d). Data measured in two years and two soil layers: 5-10 cm (a, b) and 15-20 cm (c, d).

The evidence of the effects of biochar on shape parameters can be seen through changes in patterns of the SWRCs. At 2 years after biochar application in both soil layers for the treatment with 8 Mg ha^{-1} the shape parameters α and n were significantly lower and higher than control, respectively (Table 1). Also in S2, in the upper layer 5-10 cm, for the treatment with 32 Mg ha^{-1} the parameter α was lower ($p \leq 0.10$) than the control. The SWRCs in the upper layer for the treatment with 8 and 32 Mg ha^{-1} were above that of the control treatment at matric potentials between -0.03 kPa and -33 kPa (Fig. 4). In S2, the most significant difference ($p \leq 0.05$) was for the parameter n of the treatment with 8 Mg ha^{-1} in the lower soil layer 15-20 cm. The SWRC in the lower layer for the treatment with 8 Mg ha^{-1} was above that of control at matric potentials between -1 and -10 kPa.

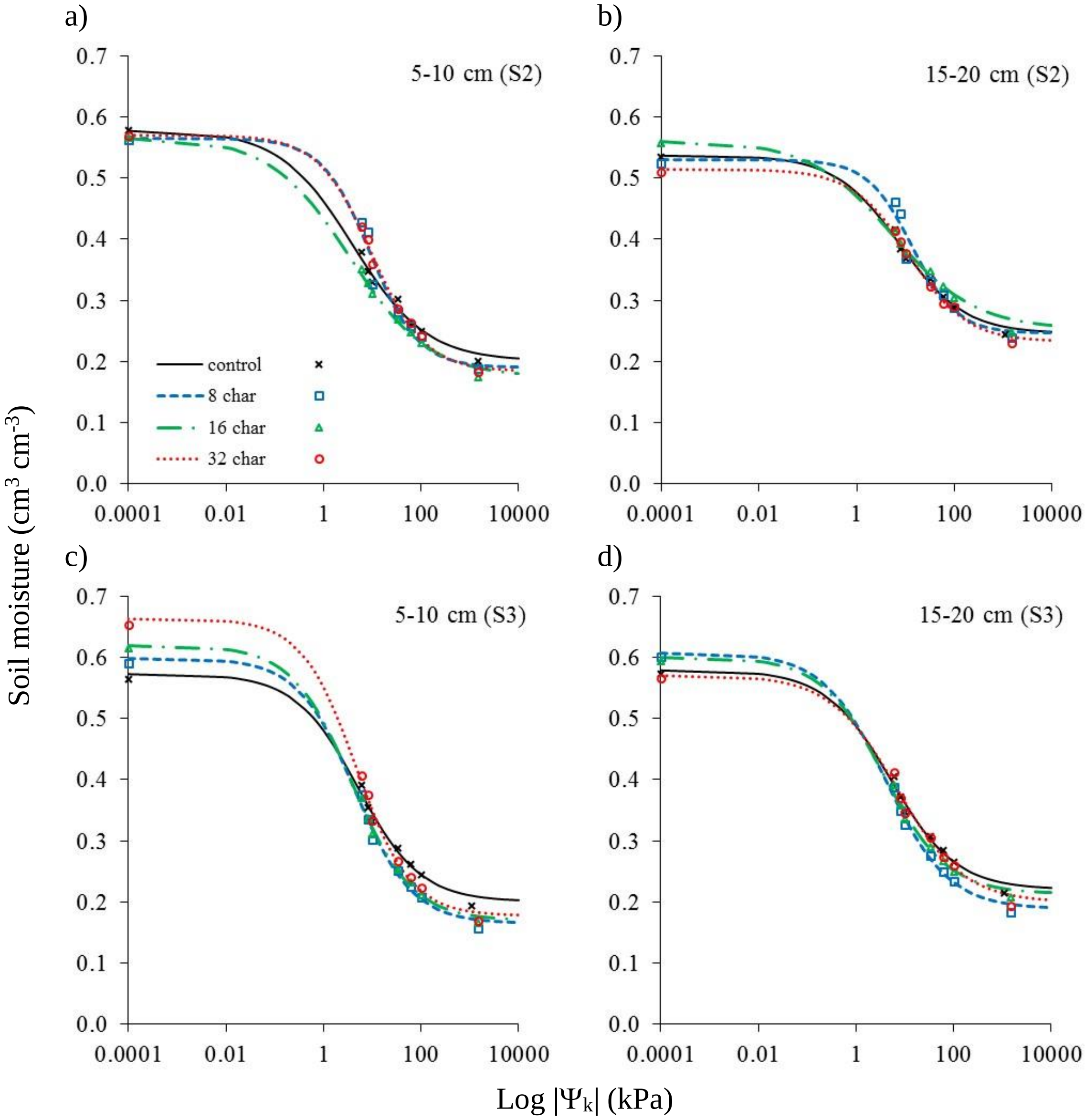

Figure 4. Predicted (lines) soil water retention curves and measured soil moisture (symbols) at a matric potential k (k = 0, -6, -8, -10, -33, -60, -100 and -1500 kPa) within 5-10 cm (a, c) and 15-20 cm (b, d) layers obtained at two (S2 - a, b) and three (S3 - c, d) years after application of biochar (8, 16 and 32 Mg ha^{-1}) in a sandy Dystric Plinthosol. Estimates of shape parameters are presented in Table 1.

In S3, no significant effects of biochar amendment on shape parameters were observed. In the upper layer, the SWRCs of the treatments with biochar amendment were all above the SWRC of the control treatment at matric potentials higher than -1 kPa, whereas at matric potentials lower than -10 kPa, the soil moisture content dropped abruptly to below that of the control treatment. This was particularly evident with 32 Mg ha^{-1}. In S3, in the lower soil layer, the same pattern was observed, but in this layer soil moisture content for treatments with biochar dropped under matric potential lower than -6 kPa, except for the SWRC of the highest biochar treatment (32 Mg ha^{-1}), which was now slightly below that of the control treatment already under matric potential higher than -1 kPa.

3.2. Response of soil physical-hydric variables to biochar application rate

Most significant responses to biochar application rate were observed in the upper soil layer (5-10 cm), with minor responses in the lower soil layer (15-20 cm; Table 2).

Table 2. Response of key physical hydric variables to biochar rate (char) at two (S2) and three (S3) years after application in a sandy Dystric Plinthosol.

Variable	Fitted model (5-10 cm)	R^2	Fitted model (15-20 cm)	R^2
		S2		
BD	1.5923	0.00	1.6388 +0.0049 char * -0.0001 char$^{2\,*}$	0.95
θ_s	0.5709	0.00	0.5395	0.00
θ_r	0.1937	0.00	0.2457	0.00
MAC	0.2006	0.00	0.1266	0.00
RAW	0.1290 +0.0013 char *	0.21	0.1234	0.00
PAW	0.1766 +0.0015 char **	0.34	0.1672	0.00
		S3		
BD	1.5651	0.00	1.6409	0.00
θ_s	0.5675 +0.0027 char ***	0.99	0.5897	0.00
θ_r	0.1785	0.00	0.2046	0.00
MAC	0.2118 +0.0019 char **	0.76	0.1919 +0.0053 char * -0.0002 char$^{2\,**}$	0.91
RAW	0.1349 +0.0013 char ***	0.89	0.1290	0.00
PAW	0.1772 +0.0013 char **	0.91	0.1698	0.00

Rate of biochar (0, 8, 16 and 32 Mg ha^{-1}). Soil bulk density (BD, g cm^{-3}), saturated soil moisture (θ_s), residual soil moisture (θ_r), macro porosity (MAC: $\hat{\theta}_0 - \hat{\theta}_6$), rice available water (RAW: $\hat{\theta}_6 - \hat{\theta}_{100}$) and plant available water (PAW: $\hat{\theta}_6 - \hat{\theta}_{1500}$). ($\hat{\theta}_k$) correspond to the soil moisture content (cm^3 cm^{-3}) at a matric potential *k*, estimated via nonlinear modeling of soil water retention curves (Fig 4). Nominal significance level of *t*-tests for the biochar effect: *** $p \leq 0.01$, ** $p \leq 0.05$, * $p \leq 0.10$; R^2: the squared Pearson correlation coefficient between measured and estimated means (n = 4).

In the upper layer, RAW and PAW increased linearly with biochar application rate. The increment in RAW and PAW was with around 1 and 0.8% for each Mg ha^{-1} of biochar applied or 21 and 17% with 1% w/w rate of biochar amendment, respectively. The response of RAW and PAW to biochar rate was stronger in S3 ($p \leq 0.05$) than in S2 ($p \leq 0.10$), with narrower confidence intervals in S3 (Fig. 5).

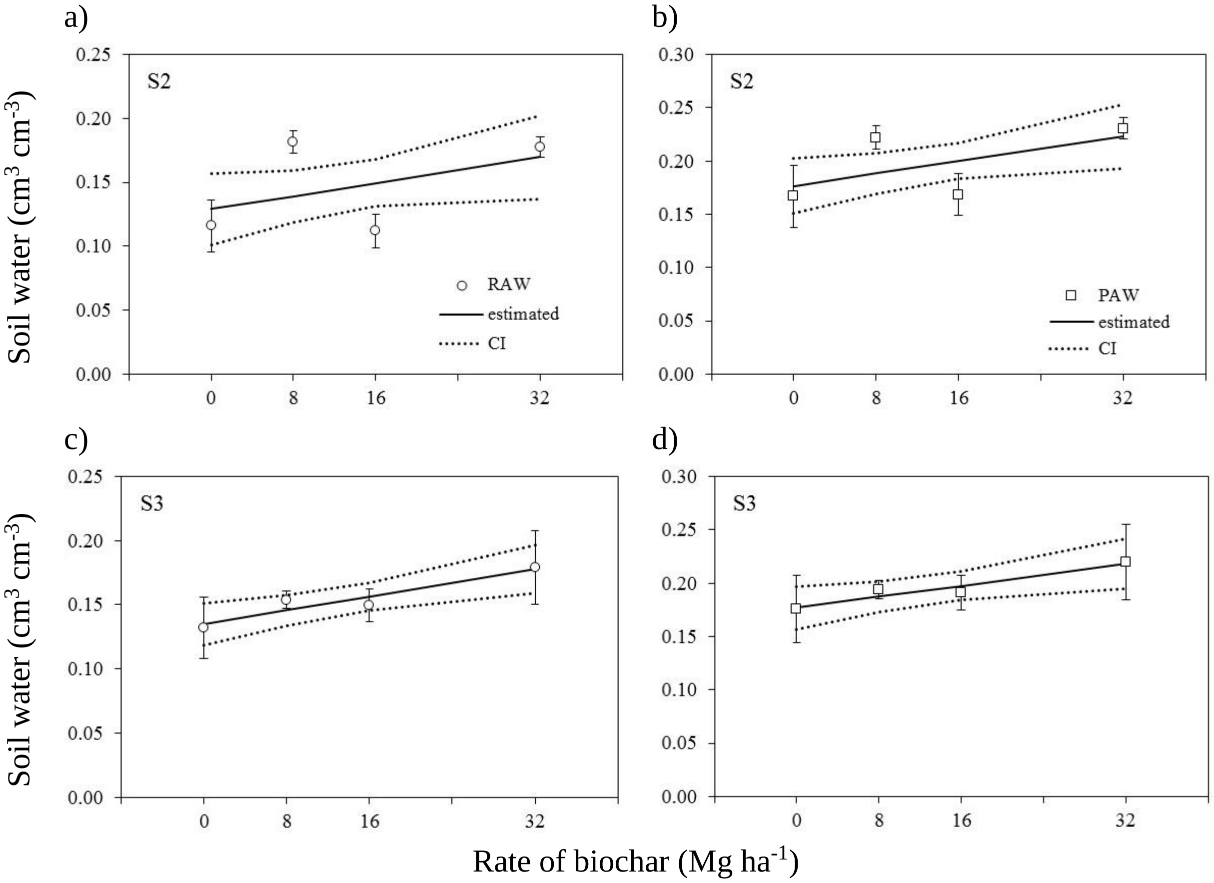

Figure 5. Rice available water (○RAW: $\hat{\theta}_6 - \hat{\theta}_{100}$) and plant available water (□PAW: $\hat{\theta}_6 - \hat{\theta}_{1500}$) in the upper 5-10 cm layer of a sandy Dystric Plinthosol at two (S2 – a, b) and three (S3 – c, d) years after application of biochar rate (0, 8, 16 and 32 Mg ha^{-1}). Symbols represent means and error bars represent standard deviation (n = 4). Solid lines represent estimated responses to biochar rate with respective 95% confidence intervals (CI, dotted lines). Parameter estimates of fitted linear models are presented in Table 2.

In S2 in the lower layer only BD was significantly affected by biochar application. The response of BD (mean ± standard error) to biochar rate followed a quadratic trend, with maximum at 16 Mg ha^{-1} (1.684 ± 0.013) and a minimum at control (1.639 ± 0.015). In S3, in the upper layer, saturated soil moisture (θ_s) and MAC increased linearly ($p \leq 0.05$) with 0.5 and 1.6% for each Mg ha^{-1} of biochar applied, respectively; whereas in the lower layer only

MAC was significantly affected by biochar application. The response of MAC to biochar rate in the lower layer followed a quadratic pattern with maximum at 16 Mg ha^{-1} (0.2299 ± 0.0152) and minimum at 32 Mg ha^{-1} (0.1744 ± 0.0184).

3.3. Response of rice yields and yield components to biochar and N application rate

There was no response of rice yields to biochar application rate in either season (Table 3).

Table 3. Response surfaces representing the effect of biochar (char) and N-fertilisation (N) rates on total shoot dry matter (TDM, Mg ha^{-1}), grain yield (GY, Mg ha^{-1}), harvest index (HI) and yield components of aerobic rice at two (S2) and three (S3) years after application in a sandy Dystric Plinthosol.

Variable	Fitted model	R^2
	------S2------	
TDM	2.10	0.00
GY	1.15	0.00
HI	0.51 +0.00172 N ** -0.00003 N$^{2\ ***}$	0.53
PAN	109 +0.9824 N *** -0.0095 N$^{2\ **}$	0.27
GP	91 -1.62735 char ** +0.04248 char$^{2\ *}$	0.18
GFI	0.81 -0.0049 char ** -0.00066 N *** +0.00014 char$^{2\ **}$	0.50
GW	25.56 -0.03206 N ***	0.32
	------S3------	
TDM	2.22 +0.0432 N *** -0.00044 N$^{2\ ***}$	0.62
GY	0.49 +0.002156 N *	0.20
HI	0.18	0.00
PAN	146 +0.8117 N ** -0.01292 N$^{2\ ***}$	0.56
GP	132	0.00
GFI	0.47 +0.00155 N ***	0.32
GW	24.99 -0.00961 N *	0.19

Rates of biochar (0, 8, 16, 32 Mg ha^{-1}) and N-fertilisation (0, 30, 60, 90 kg ha^{-1}). PAN: number of panicles m^{-2}; GP: number of grains panicle^{-1}; GFI: grain filling index; GW: 1000-grain weight (g). Nominal significance level of *t*-tests for the effects of biochar and N: *** $p \leq 0.01$, ** $p \leq 0.05$, * $p \leq 0.10$; R^2: the Pearson correlation coefficient between observed and estimated means (n = 16).

In S2, total shoot dry matter (TDM) and grain yield (GY) were not affected by biochar or N application rate. Both TDM and GY varied greatly, from 0.57 and 0.17 Mg ha^{-1} (with 32 Mg ha^{-1} and without N) to 4.04 and 1.99 Mg ha^{-1} (with 32 Mg ha^{-1} and 90 kg N ha^{-1}), respectively. Most significant ($p \leq 0.05$) effects of biochar were observed on number of grains panicle^{-1} (GP) and grain filling index (GFI). The response of GP and GFI to biochar rate followed a quadratic pattern with a minimum obtained at about 16 Mg ha^{-1}. The response of harvest index (HI) and number of panicles m^{-2} (PAN) to N rate followed a quadratic pattern with a maximum at around 30 to 60 kg N ha^{-1}. The estimated HI (mean ± standard error) varied from a minimum at 0.42 ± 0.02 (with 90 kg N ha^{-1}) to a maximum at 0.53 ± 0.02 (with 30 kg N ha^{-1}) and PAN from 109 ± 7 (without N) to 133 ± 5 (with 60 kg N ha^{-1}). The GFI and 1000-grain weight (GW) decreased with increasing N rate.

A year later, in S3, the effect of biochar on any characteristic measured at crop maturity of rice was totally absent. The response of TDM and PAN to N rate followed a quadratic pattern with a maximum at 30 to 60 kg N ha^{-1}, whereas GY and GFI increased linearly with increasing N rate. Estimated GY increased from 0.49 ± 0.2 Mg ha^{-1} (without N) to 0.69 ± 0.2 Mg ha^{-1} (90 kg N ha^{-1}), regardless of biochar application (Table 3). The observed GY varied from 0.38 (with 8 Mg ha^{-1} and without N) to 0.93 Mg ha^{-1} (with 16 Mg ha^{-1} and 60 kg N ha^{-1}). The HI and GP were not affected by N treatments, whereas GW decreased linearly ($p \leq 0.10$) with increasing N rate. The GY in both seasons were rather low, mainly due to weed infestations. Chemical and mechanical controls were applied when necessary, but these could not sufficiently compensate for the low resistance of the cultivar BRS Primavera to biotic stresses.

4. Discussion

Here we summarize and discuss the main findings of this study as follows: i) the impact of the wood biochar application rate on WRC of the sandy loam Dystric Plinthosol is positive and persistent at 2 and 3 years after application; ii) although soil WRC increases with biochar application rate, we did not observe any impact on rice yield; and iii) the proposed nonlinear mixed (NLM) model was an innovative analytical tool for such a large field trial.

Our results showed that in both seasons PAW and RAW in the upper 5-10 cm layer of the sandy loam soil increased proportionally to biochar application rate with about 0.8 and 1% for each Mg ha^{-1} of biochar applied, respectively (Fig. 5). The consistent increase in soil WRC seems to be related to a slight increase in soil moisture at -6 kPa for the treatment with 32 Mg ha^{-1}, as can be observed by means of SWRCs in S2 and S3 (Fig. 4), with a significant effect on the shape parameter α in S2 (Table 1). In S2 we also observed significant changes in shape parameters of the SWRC with 8 Mg ha^{-1} (Table 1). However, there was no such effect for the treatment with 16 Mg ha^{-1}, where the increase in soil WRC seems to be a consequence of a decrease in soil moisture content with biochar rate up to 16 Mg ha^{-1} at matric potentials of -100 kPa ($p \leq 0.13$) and -1500 kPa ($p \leq 0.16$) in S2. The uncertainty of the linear response of PAW and RAW to biochar rate was higher in S2 than in S3, predominantly for rates of 8 and 16 Mg ha^{-1} (Fig. 5). The uncertainty can be related to changes in BD affecting the overall response to biochar application. In fact, BD was generally 1.7% higher in S2 than in S3 (Table 2), which was a consequence of mechanical weeding using a tractor which passed twice over all plots of the field trial just prior to sowing in S2. Even though we observed no effect of biochar rate on BD in the upper soil layer, in the lower soil layer 15-20 cm BD increased with biochar rate up to 16 Mg ha^{-1}.

At matric potentials lower than -8 kPa the amount of water in soils treated with biochar decreased abruptly in both years while in S3 in the upper soil layer θ_s and MAC increased significantly with increasing biochar rate (Table 2). It seems that biochar application lead to an increase in soil moisture at a matric potential up to around -6 and -8 kPa that was not necessarily sustained under lower matric potentials (Fig. 4). Therefore, the effect of biochar on soil WRC is most likely a consequence of an effect in overall porosity of the soil. We found a notable increase in MAC of 51% with 1.5% w/w biochar amendment. The increase in MAC with biochar application rate was mostly related to the large particle size ($\leq$ 2000 μm) of the biochar tested. For instance, Abel et al. (2013) reported an increase of 15% in total porosity and 6% in air capacity with application of 5% w/w beech wood biochar (particle sized < 5000 μm) that lead to a 35% increase in PAW in a loamy sand soil. According to the van Genuchten model described by Ibrahim et al. (2013), there was an 8% increase in PAW with application of 1.5% w/w very fine particle sized biochar (44-149 μm) in a sandy loam soil. Additionally, the SWRCs that they modelled indicate a greater impact on soil WRC at low matric potentials. However, application of fine particle size material under field conditions is difficult since it is easily moved by wind. Combination of biochar with liquid or solid fertilisers could be an option to avoid such kind of losses and capture the potential positive effect of biochar on soil WRC. Liu et al. (2012a), for example, observed that application of 20 Mg ha^{-1} of biochar with 50 Mg ha^{-1} of organic compost has a more prominent positive effect on water availability than application of pure compost.

The biochar we applied in the field trial is a by-product of slow pyrolysis (under ~ 450 ºC) of eucalyptus wood, which resulted in a material with an intrinsic porosity ≤ 10 μm (Fig. 2) and a relatively much lower SSA (3.2 m^2 g^{-1}) if compared to a wood biochar produced under greater temperature of pyrolysis, such as the one tested by Dempster et al. (2012). They observed an astonishing increment in volumetric soil moisture content at very low matric potentials of -100 and -1500 kPa by 71 and 127%, respectively; with application of 1.8% w/w biochar (SSA 273 m^2 g^{-1}) artificially packed with a sandy soil. Logically a higher SSA biochar has more and finer pores and therefore a greater effect on soil WRC, as demonstrated by Lei and Zhang (2013). They observed a tremendous increase in soil water content between -33 and -1500 kPa in a sandy loam soil treated with 5% w/w woodchip biochar pyrolysized at 300, 500 and 700 ºC (SSA 24, 67 and 124 m^2 g^{-1}) of 39, 51 and 55%, respectively. We found a rise of 6, 13 and 26% in PAW, accompanied by a 4, 8 and 16% increase in θ_s with 0.4, 0.7, and 1.5% w/w biochar, respectively (Table 2). Relatively, the increase in θ_s is much higher than the 0.2% increase with 1% w/w biochar observed by Abel et al. (2013). The rise in PAW that we found, though, is lower than the 28% rise observed by Abel et al. (2013), and higher than the 6% rise found by Ibrahim et al. (2013) with 1% w/w biochar. Apart from differences in time after application and conditions of experimental setup, SSA of the biochar used is probably the main cause for these discrepancies. However, neither Abel et al. (2013) nor Ibrahim et al. (2013) determined the SSA of the biochar they used. High resolution images indicate that there are differences in the pore structure of the beech wood biochar used by Abel et al. (2013) and the one used in our study (Fig. 2). The SSA of the biochar we used is similar to the birch wood biochar (particle sized < 10000 μm) used by Karhu et al. (2011) but lower than the SSA of the eucalyptus biochar produced at 450 ºC (milled to powder) described by Borneman et al. (2007). Karhu et al. (2011) observed an effect in gravimetric soil moisture at 0 kPa relatively higher than the effect we observed on volumetric soil moisture at 0 kPa (θ_s) with application of 0.3% w/w biochar.

Beyond the influence that both SSA and particle size of biochar have *per se* on the soil WRC of a sandy soil, we must also consider the application rate. The maximum rate applied in our study was of 1.5% w/w, which is half of the minimum rate (3% w/w) used in other studies that have shown great impact of biochar on soil WRC of sandy soils under artificially controlled conditions (Pereira et al. 2012, Basso et al. 2013). For instance, Basso et al. (2013) found a spectacular increase in available water content between -10 and -1500 kPa of 44 and 38% with application of 3 and 6% w/w fast pyrolysis red oak biochar, respectively. Feasibility of application of such high rates in agricultural fields should be assessed regionally. The highest rate applied in our study is already pushing the limits for practical applications. For example, according to Filiberto and Gaunt (2013), assuming yield increase and fertiliser savings, the costs for application of 25 Mg ha^{-1} biochar rate in agricultural fields may not be economically feasible. Besides the differences in the rate of biochar used, studies are frequently conducted under artificially controlled conditions and did not evaluate the effect on plant biomass. One of the exceptions is Asai et al. (2009), who tested the effect of a wood residue biochar on saturated hydraulic conductivity accompanied by measurements on rice yield. They found an increase in saturated hydraulic conductivity with application of 16 Mg ha^{-1} biochar in the 0-5 cm surface of a silt loam soil, but no effect on rice yield.

According to a meta-analysis done by Jeffery et al. (2011) biochar application generally leads to a 10% increase in crop yields, although causes are poorly quantified and effects differ between crops. Likewise, Liu et al. (2013) found via a meta-analysis an average increase in crop productivity by 11% with biochar amendment, with greater crop responses in pot than in field experiments. We observed no response of rice GY to biochar application rate during both seasons under assessment (Table 3). Yet, in S2, GFI and GP, which are yield components strongly sensitive to water stress (Fageria, 2001), increased with biochar rate higher than 16 Mg ha^{-1}. In rain fed systems soil matric potential can drop below -100 kPa any time during the growing season. The associated water stress leads to a reduction in overall water use efficiency by rice (Wopereis et al., 1996). Therefore, we defined rice stress free available water content as the soil moisture content between -6 and -100 kPa (RAW). We observed an increase of 32% in RAW with the addition of 32 Mg ha^{-1}, which is equivalent to 17 to 18 mm in the upper 5-10 cm layer of the sandy loam soil. This additional amount of water would be sufficient to satisfy the crop demand for approximately 4 additional days without rainfall, considering that the evapotranspiration rate in an uncovered soil is ca. 5 ± 0.5 mm day^{-1} during the critical stage of rice production under aerobic conditions (around 45 to 75 DAE) in the Brazilian savannah (Stone and Moreira, 2005). During the critical period for rice production in seasons under assessment in this study, in Jan./Feb. 2011 (S2) and Jan./Feb. 2012 (S3), the amount of rainfall was high (~ 650 mm) and twice the amount during the critical period in previous seasons, in Feb./Mar. 2009 and Jan./Feb. 2010 (Fig. 1). If there is a positive effect of biochar on RAW of the sandy loam soil, then the effect on rice GY would be a consequence of lower precipitation rate, such as in the first seasons of the trial reported by Petter et al. (2012). Throughout the latest 35 years (from 1979 to 2013) average precipitation rate during the months of January and February in the municipality where the field trial is located was 507 mm and the frequency of an amount of rainfall lower than 650 mm was 74% (Agritempo, 2014). In other words, in this region of Brazil’s tropical savannah, rainfall during the critical period for rice production is frequently lower than 650 mm. This means that application of biochar could be sound agronomic practice that could reduce water stress and improve yield stability. If the application of biochar can optimize water use, then increased rice productivity seems a conceivable result, especially for farmers relying in scarce resources under tropical and subtropical regions (Nabahungu and Visser, 2013; Masood et al., 2014). Furthermore, salinization is a worrying subject in rice production areas in arid regions of the world (Ahmad et al., 2013; Ghafoor et al., 2012) and an increase in soil moisture with wood biochar application can potentially help to reduce this problem.

Finally, we have demonstrated that NLM can be used as an innovative analytical tool to model SWRC and compare the shape parameters α and n via formal tests. By using a NLM model, we were able to account for the random effect of latent variables related to measurements taken in the same sample unit within a specific location (plot), leading to a reduction in the uncertainty of estimation of the SWRCs (Fig. 3). By using the SAS program, results of model fitting were generated by year and soil layer in one run, facilitating the management of the large data set.

5. Conclusions

We found a consistent increase in plant available water and rice available water in the upper soil layer of around 0.8% and 1% for each Mg ha^{-1} biochar amendment, respectively, 2 and 3 years after its application. The impact on water retention capacity of the sandy loam soil is mostly related to an effect in overall porosity of the soil matrix and did not result in increased rice yield, most likely because, in both seasons under study, rainfall during critical period for rice production exceeded 650 mm. The use of biochar as a soil amendment could be a worthwhile strategy to improve yield stability under water limited seasons. These findings call for longer term field trials with feasible amounts of biochar application, which are usually lower than those applied in artificially controlled studies. In addition, detailed analyses of all biochar properties should become a standard procedure in order to better target its use as a soil amendment; different sources and methods of creating biochar can lead to very different char properties. Important properties to be reported are specific surface area and particle size. Expression of the rate of biochar on a dry mass basis can also facilitate comparison of findings. In addition to our main findings, we also demonstrated the utility and adequacy of the nonlinear mixed modelling to make statistical inferences on SWRCs by accounting for spatial variability and expected dependencies arising from measurements taken in the same sample unit within a specific plot in the field trial.

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Chapter 4

Chemical and physical properties of a clay soil along 3.5 years after biochar application and the impact on rice yield

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"Closing the gap between actual and attainable resource use efficiencies should become as important as closing yield gaps."

(H. Spiertz, Europ. J. Agronomy, 43:1-8, 2012)

Abstract

We assessed the impact of a single application of biochar on soil chemical and physical properties and aerobic rice grain yield on irrigated kaolinitic clay Ferralsol in a tropical Savannah. We used linear mixed models to analyse the response of soil and plant variables to biochar rates (0, 8, 16, 32 t ha^{-1}) and mineral N fertilization rates (0, 30, 60, 90 kg N ha^{-1}), and their interaction. The response was analysed within three growing seasons of aerobic rice (S1.5, S2.5 and S3.5), equivalent to 1.5, 2.5 and 3.5 years after biochar application. Soil organic matter increased with biochar rate, irrespective of N fertilization, at S2.5 and S3.5, whereas rice stress free available water decreased with biochar rate at S1.5 and S2.5. Rice grain yield and yield components varied with the seasons according to the changes in soil properties and weather conditions. A single application of biochar up to 32 t ha^{-1} (1.6% w/w) was insufficient for ensuring aerobic rice yield increase and stability on kaolinitic clay Ferralsol under the climatic conditions of the Brazilian Savannah prone to dry spells. Most likely, the positive effects of biochar on soil chemical properties on rice production were offset by its negative effects on soil water retention capacity and N uptake by the crop.

Keywords: carbonised biomass, soil organic matter, soil water retention capacity

1. Introduction

Approximately 3 billion people depend on rice as a staple food worldwide (Nguyen, 2002). In Brazil, total rice consumption is 11 million t annually (IBGE, 2009). In March 2012, the total area of rice grown in Brazil was of 2.5 million ha, of which around 44% was covered by aerobic systems (IBGE, 2012). Aerobic rice is typically grown on well-drained soils, without surface water accumulation (Fageria, 2001). A consistent decline in the total area of aerobic rice has been observed since the mid 80's (Pinheiro et al., 2006), and in the 2011/12 growing season alone a decline in area as large as 13% was reported (Conab, 2012). The key reason for a decline in aerobic rice area is the greatly variable yield, ranging from 1 to 5 t ha^{-1}, caused by high rainfall variability with frequent, extended dry spells (Conab, 2012). Low productivity is a concern, even though high-yielding aerobic rice varieties are available throughout Brazil (Breseghello et al., 2011).

In the Brazilian Central West region, where about 38% of the rice production area is located (IBGE, 2012), the predominant biome is a tropical Savannah, characterized by acid-dystrophic soil types. In this region, low aerobic rice yield is not only a result of water stress, which can cause severe damage during the reproductive phase (Heinemann et al., 2011), but is also a consequence of the low N recovery of this plant (Fageria, 2001). Although low competitiveness of rice with weeds and high incidence of rice blast (*Magnaporthe grisea*) also has a profound effect, it is evident that improving soil fertility is key for overcoming low productivity (Fageria, 2001). The soil fertility has three components (physical, chemical and biological), which are dependent on the inherent characteristics of the soil and on the management practices implemented (Abbott and Murphy, 2003).

The use of biochar as soil amendment might increase crop yields by increasing soil pH and soil water retention capacity (Jeffery et al., 2011). This, in turn, can increase nutrient availability. Biochar is the charred by-product of biomass pyrolysis. Various organic materials, like agricultural by-products (rice husks, peanut shells, coconut shells, wheat straw), biomass energy crops (wood, sugarcane straw), green waste, animal waste, kitchen waste and sewage sludge can all be processed by pyrolysis resulting in synthetic gas, liquid bio-oil and solid biochar (Sohi et al., 2010). The usefulness of biochar in agriculture depends on its composition and availability. We tested a by-product of charcoal production from plantation timber, which is readily available in the region where this study was conducted. Like most wood biochar, this type of biochar has a high concentration of resistant C leading to a significant residence time in soils, a desirable characteristic under conditions of poor soil fertility and high mineralization rate of soil organic matter, such as in tropical Savannahs. The wood biochar used in this study is also rich in micro pores (Fig. 1), which can lead to changes in soil water retention capacity (WRC). However, under field conditions, Major et al. (2012) found no effect on WRC of a Ferralsol (40-44% clay) with application of 3% w/w wood biochar. Likewise, Asai et al. (2009) found no effect on WRC of a 48% clay soil with application of 1.2% w/w wood biochar. Significant effects of biochar on WRC of clay soils were observed with much greater rates (Tryon, 1948; Chen et al., 2010; Fellet et al., 2011; Kameyama et al., 2012; Pudasaini et al., 2012). Tryon (1948) observed negative effects on physical hydric properties of a clay loam soil with 15% (by volume) wood biochar amendment. To the contrary, Fellet et al. (2011) found significant increase in WRC of an 83% clay soil with 5 and 10% w/w prune residue biochar. Similarly, Kameyama et al. (2012) found an increase in WRC with increasing sugarcane bagasse biochar rate (from 1 to 10% w/w) but only under a matric potential higher than -10 kPa and Chen et al. (2010) observed a 39% increment in available soil moisture with 3% bagasse biochar application in a heavy clay soil. Pudasaini et al. (2012) only found significant increase in WRC in a clay Ferralsol treated with massive rates of 40% and 60% w/w green waste biochar. These divergent results on the effect of biochar on WRC of a clay soil call for clarification. Apart from biochar rates and porosity, cultivation and soil chemical properties can also influence the response of soil physical properties to biochar application, given that continuity of pores and aggregation are relevant to hydraulic behaviour of well-structured clay soils. The inherent high spatial variability under field conditions is also a constraint that should be accounted for estimation of soil WRC (Hardie et al., 2014; Omuto et al., 2006).

Despite the stability of biochar relative to other forms of carbon, it undergoes physical and biological oxidation, fragmentation, and carboxylation when applied to the soil (Kookana et al., 2011). The influences of biochar application on chemical and physical soil properties and, consequently on crop yields, may change over time due to the biogeochemical interactions that occur in the soil. To understand better these interactions, longer-term field trials are necessary. Some studies conducted in cropping systems have shown lasting positive effects of biochar on crop yields and soil chemical properties. Even 4 years after incorporation of biochar into a sandy clay loam Ferralsol, soybean yields were still significantly increased (Madari et al., 2010 cited by Maia et al., 2011). On a Colombian Ferralsol, there was no immediate effect, but in the second to fourth season after application of 20 t ha^{-1} wood biochar maize yields were consistently higher (Major et al., 2010). So far, there are no studies showing how a single application of biochar might affect aerobic rice yields over a longer period in a tropical Savannah. One weakness raised in the meta-analysis by Jeffery et al. (2011) is that most of the available field data were on effects at 1 or 2 years after application. Hence, longer-term data is required, particularly concerning effects of soil cultivation on biochar. Likewise, in a meta-analysis by Liu et al. (2013) they highlighted the importance of field studies, especially because crop response to biochar application could be overestimated under artificially controlled conditions.

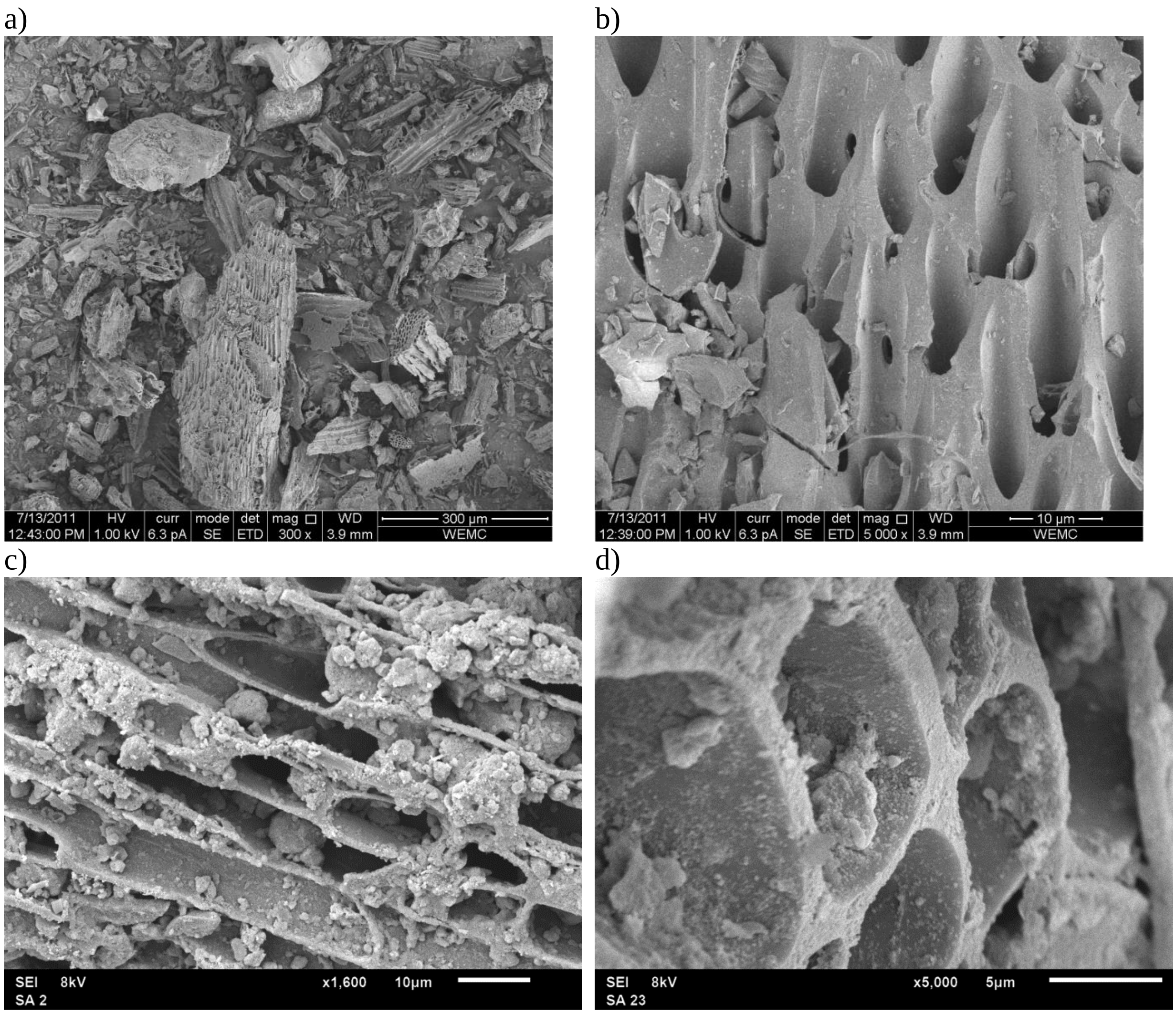

Figure 1. Scanning electron microscopy (SEM) images of wood biochar milled to pass through a 2000 μm sieve before (a, b) and 1.5 years after incorporation to a clay Ferralsol (c, d). The SEM images of biochar were made using sub-nanometer resolution with FEI Magellan 400 (Fig. 1 a, b) and Jeol, JSM – 6610 (Fig. 1 c, d) equipments.

Therefore, aiming to assess the effect of biochar application under field conditions, we studied the impact of wood biochar along three growing seasons of aerobic rice. This study is a follow up of the study by Carvalho et al. (2013). They observed negative effects on plant growth and no yield response in the first rice season following biochar application, although positive effects on soil chemical properties were present. Here we tested the following hypotheses: i) there is a lasting positive effect of biochar on soil chemical properties; ii) since the wood biochar is rich in C, and about 6% of this C is labile, application of high amounts (> 16 t ha^{-1}) results in a temporary decrease in N availability to the crop; iii) positive effects of biochar rate on rice grain yield depends on adequate N fertilization; and iv) application of biochar in the cropping system can negatively affect the physical properties of the clay soil. The aim of this study was to investigate the effects of a single application of wood biochar combined with mineral N fertilization on soil chemical and physical properties and on aerobic rice grain yield at time intervals of 1.5, 2.5 and 3.5 years after biochar application in an irrigated clay Ferralsol.

2. Materials and methods

2.1. Experimental setup and agronomic management

An experiment under centre pivot irrigation was installed in June 2009 on a kaolinitic clay Rhodic Ferralsol at Embrapa Rice and Beans, Capivara Farm, in Santo Antônio de Goiás, Goiás State, Central West region of Brazil (16°29'17 "S and 49°17'57 "W). Since 2001, the area had been cultivated under no-tillage with an intercrop of corn (*Zea mays*) with grass (*Urochloa ruziziensis*) in summer (November to February), followed by irrigated common bean (*Phaseolus vulgaris*) in winter (June to August). Immediately after establishment of the field trial, irrigated common bean was cultivated as the first crop in winter, followed by rice (*Oryza sativa*) in summer. This was repeated for subsequent cropping seasons, except that a third crop, rice from March to June in 2010 and millet (*Pennisetum glaucum*) from March to June in 2011 and 2012, was grown in between rice and common bean. At establishment of the field trial, the soil was ploughed twice to a depth of 0-20 cm in order to incorporate crop residues and guarantee uniform incorporation of biochar into the soil. The biochar was applied only once, June 9, 2009. Biochar was milled to pass through a 2000-μm sieve, spread manually over the soil surface, and incorporated to a depth of approximately 10-15 cm using a harrow. Sixty-four experimental plots of 40 m^2 were arranged in four blocks with combinations of biochar rates (0, 8, 16, 32 t ha^{-1}) and mineral N fertilizer (0, 30, 60, 90 kg ha^{-1}). Plots had to be placed out of the way of the pivot wheels, resulting in one incomplete block. Biochar rates were applied only once, randomly distributed within each block. The same N rate was applied in sequential strips across the four blocks every growing season. In this paper we report on the second (08/11/10 to 21/02/11), third (28/11/11 to 19/03/12) and fourth (17/11/12 to 25/03/13) growing seasons of rice as the main crop, corresponding to 1.5 (S1.5), 2.5 (S2.5) and 3.5 (S3.5) years after biochar application to the soil. The first growing season of rice (03/11/09 to 22/02/10), corresponding to 0.5 year after biochar application, was reported by Carvalho et al. (2013).

We used BRS Primavera, an aerobic rice cultivar released in 1997 as one of the first to present desirable grain quality for Brazilian rice processing industry and consumers: long slender kernels, and non-stick cooking quality (Breseghello et al., 2011). It has an attainable yield of ca. 4.4 t ha^{-1}, however it is susceptible to rice blast and lodging (under highly fertile soil conditions), with low tolerance to drought stress (Pinheiro et al., 2006). Rice was sown at 110 seeds m^{-1}, with a 5-row Semeato® direct sower adapted for no-tillage systems, with 40-cm row spacing. Before the beginning of each growing season, a 500-g sample of soil was collected from 2 points within each plot at a depth of 0-20 cm. The P and K availability were determined using a Mehlich 1 solution (Mehlich, 1953) for extraction followed by atomic absorption spectrometry determination according to Embrapa (2009). The rate of P and K application was calculated based on the average availability of all plots and on recommendations for aerobic rice systems (Embrapa, 2007). Every plot was given the same rate of P-K (kg ha^{-1}) at sowing in S1.5 (60-30), in S2.5 (30-30), and in S3.5 (40-40). Mineral N (urea) was divided into three applications: at sowing, and at 25 and 45 days after emergence (DAE). Rice blast was monitored and controlled when necessary with two sprayings of Bin® (300 g ha^{-1}) around 45 and 55 DAE. Weeds infestation was chemically controlled with either Glyphosate® (5 L ha^{-1}) or Gramoxone® (1 L ha^{-1}) around 15 days prior sowing and with either Star Rice® (0.4 L ha^{-1}) or 2-4D (0.7 L ha^{-1}) around 10 DAE; together with hand weeding around 45 and 75 DAE.

Monthly precipitation, irrigation and average maximum and minimum temperatures throughout growing seasons are presented in Fig. 2 based on Embrapa Rice and Beans' weather station database available at Agritempo (2014). In order to avoid complete crop failure, irrigation was applied by sprinklers only if consecutive 6 days without rain occurred.

In S1.5 the amount of irrigation was 9 mm in November (after sowing), 23 mm in January (around 60-75 DAE), and 9 mm in February (around 86-92 DAE); in S2.5 only 9 mm was applied in February (around 90 DAE) and in S3.5 no irrigation was applied.

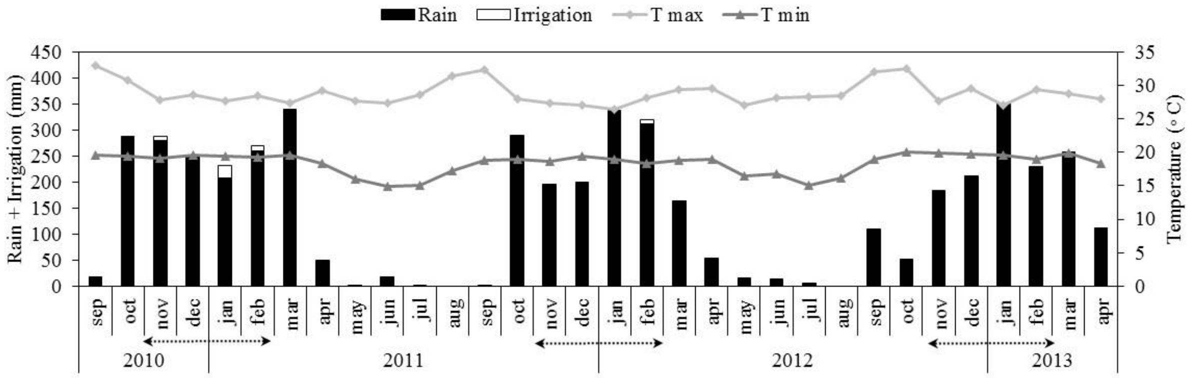

Figure 2. Monthly precipitation (rain), irrigation and average of maximum (T max) and minimum (T min) temperatures at growing seasons 2010/11, 2011/12 and 2012/13, corresponding to 1.5, 2.5 and 3.5 years after application of biochar into a clay Rhodic Ferralsol at in Santo Antônio de Goiás, GO, Brazil. Dotted arrows indicate the experimental period, when aerobic rice was grown.

2.2. Initial chemical and physical properties of soil and biochar

At the beginning of the 2010/11 season, soil chemical properties (to a depth of 20 cm) were determined in control plots without biochar and N fertilization as follows: soil organic matter (SOM) using the Walkley-Black method (Nelson and Sommers, 1996); pH using a 1:1 soil:water solution (Bates, 1973); Ca, Mg and Al were extracted using a 1 mol L^{-1} KCl solution (Gavlak et al., 2003); K and P were extracted using a Mehlich 1 solution (Mehlich, 1953). Extraction was followed by determination via atomic absorption spectrometry (Embrapa, 2009). The texture of the soil was determined using a standard hydrometer and the Bouyoucos scale (Gee and Bauder, 1986). The same methods used to analyse chemical properties of soil were used to determine chemical properties of biochar. The ash content and functional groups of biochar were determined as follows, respectively: i) for reactive ashes removal, around 7-g sample of biochar was treated with hychloridric acid (HCl) 0.05 mol dm^{-3}, shaken for 24 h, followed by washing with $CaCl_2$ 1 mol dm^{-3} twice and washed four times with deionized water until pH ~ 5. Biochar samples were dried at 50 ºC in a mechanical convection oven for 60 h (Fidel 2013); ii) the surface functional groups were determined by reaction with Boehm reactants. Following reactive ashes removal, 0.5-g biochar was reacted with 25 mL of three Boehm alkaline solutions ($NaHCO_3$ 0.05 mol dm^{-3}, Na_2CO_3 0.05 mol dm^{-3} and NaOH 0.05 mol dm^{-3}) for 24 h. Solutions were filtered with 0.45 µm cellulose ester membrane, and the pH of 10 mL aliquots were lowered by adding 20 mL of HCl 0.05 mol dm^{-3} to $NaHCO_3$ and NaOH extracts and 30 mL to Na_2CO_3 extracts. The CO_2 was removed by sparkling with N_2 (Fidel, 2013) before titration with 0.05 mol dm^{-3} Sodium Tetra borate solution (Vogel, 1989). Properties of biochar and chemical properties of the soil are presented in Table 1. A single point surface area of biochar was determined by the Brunauer, Emmelet and Teller (BET) nitrogen absorption method (Brunauer et al., 1938), using nitrogen gas sorption analysis at 77.3 K (-195.9 ºC). Pore structure of the biochar is shown in Fig. 1. The scanning electron microscope (SEM) images of biochar were made using sub-nanometer resolution with FEI Magellan 400 (Fig. 1 a, b) and Jeol, JSM – 6610 (Fig. 1 c, d) equipments.

Table 1. Properties of the clay Rhodic Ferralsol and wood biochar.

Properties	Clay Soil	Biochar
Clay (g kg^{-1})	574	-
Sand (g kg^{-1})	326	-
Silt (g kg^{-1})	100	-
SOM (g kg^{-1})	24	-
Ca (mmol$_c$ kg^{-1})	11	25
Mg (mmol$_c$ kg^{-1})	4	8
Al (mmol$_c$ kg^{-1})	3	0
P (mg kg^{-1})	28	72
K (mg kg^{-1})	75	780
pH (H_2O)	5.4	7.6
pH ($CaCl_2$) 0.01M	-	6.4
C (% weight)	-	75.89
N (% weight)	-	0.78
Oxidisable C (% weight)	-	5.89
Lactone groups (meq g^{-1})	-	0.16
Phenol groups (meq g^{-1})	-	0.35
Carboxyl groups (meq g^{-1})	-	0.41
Ash content (meq g^{-1})	-	0.45
SSA (m^2 g^{-1})	-	32.40

SOM: soil organic matter; SSA: specific surface area.

2.3. Measurements of soil chemical properties and plant response

At 1.5, 2.5 and 3.5 years after biochar application, around 75 DAE, 3 soil sub-samples of 500 g at 0-20 cm soil depth were collected from each plot (between rows of rice) to determine soil chemical properties. Soil samples were air dried and milled to pass through a 2000-μm sieve. Soil organic matter (SOM), pH, Ca, Mg, Al, P and K were determined using the same methods for soil analysis described in section 2.2. The cation exchange capacity (CEC) was calculated as the sum of Ca, Mg, Al and K.

At crop maturity, around 100 DAE, total shoot dry matter, grain yield (weight of rice grains dried to 13% moisture) and yield components (number of panicles, grains per panicle, grain filling index and 1000-grain weight) were determined in samples collected from 2 rows of 3-m length in the centre of each plot. Harvest index was calculated as the ratio between grain yield and total shoot dry matter. Filled and unfilled grains from panicles within the harvested area were separated with a vertical blower and counted with a seed counter. Grain filling index was calculated as the ratio between the number of filled grains and the total number of grains. Just before the harvest, around 94 DAE, a fixed number of panicles were collected in each plot to determine the number of panicles infected by rice blast (*Magnaporthe grisea*). The number of infected panicles was visually determined.

Additionally, in S1.5 and S2.5 we were able to determine concentration of N in grains. Concentration of N in the grain was measured in a 10-g sub-sample collected from the sample used to quantify grain yield. Rice grains were washed with deionized water, dried (75 °C for 48 hours), weighed and milled to pass through a 200-μm sieve. Samples of around 3.5 mg were analysed in a PerkinElmer 2400 Series II CHNS/O Elemental Analyser (Nelson and Sommers, 1996).

To analyse the data we used linear mixed models, which allowed us to account for potential spatial autocorrelation among plot measurements. Location of a plot was established by its position in a specific column and row on the field trial. Rows and columns were included as random effects, and linear effects of biochar and N, its interaction, and corresponding quadratic terms were included as fixed effects in the model. Model parameters were estimated by restricted maximum likelihood – REML. Analyses were performed using the MIXED procedure (Proc MIXED) of the statistical software SAS/STAT® (SAS Institute Inc. 2008). Complete mixed model analysis of the final model, including residual analysis, influential diagnostics and checking for potential model violations was conducted using the ODS GRAPHICS option.

Surfaces for identifying patterns of response of plant and soil variables to biochar and N treatments were estimated for each season separately. A complete quadratic response surface (Eq. 1) in which all predictors (biochar, N and biochar×N) were included was the starting point:

$$y_{ijcr} = \beta_0 + \beta_1 char_i + \beta_2 N_i + \beta_3 char_i * N_i + \beta_4 char_i^2 + \beta_5 N_i^2 + c_c + d_r + e_{ijcr} \qquad \text{(Eq. 1)},$$

where y_{ijcr} is the observation of the response variable y corresponding to biochar and N treatments i (i = 1, 2, 3, 4, …, 16) of the replication j (j = 1, 2, 3, 4); β_0 is the intercept; β_1 and β_2 are the linear effects of biochar and N, respectively; β_3 is the interaction effect biochar×N; β_4 and β_5 are the quadratic effects of biochar and N, respectively; c_c and $d_r \sim N(0, \Sigma)$, random effects to account for potential spatial effect related to localization of a plot in a column c (c = column 1, …, column 9) and in a row r (r = row 1, …, row 11); and $e_{ijcr} \sim N(0, \sigma^2)$, the random error associated to each observation y_{ijcr}.

To determine the appropriate response surface, predictors containing the highest p-value ($p > 0.10$) were progressively excluded. Linear terms were retained whenever interaction or quadratic terms were significant (MacCullagh and Nelder, 1983). Due to the large experimental area, relatively high residual variances were anticipated and for that reason, 0.10 was chosen as the appropriate significance level in the process of reducing the response surface model to safeguard against high type II error. The magnitude of evidence for the remaining effects was assessed by nominal significance levels. The model goodness-of-fit was summarized via the squared Pearson correlation coefficient between observed and predicted values.

2.4. Measurements of soil physical properties

The soil water retention capacity (WRC) was evaluated at 1.5 (S1.5) and 2.5 (S2.5) years after biochar application. Soil samples (cylinders of inox steel of 5 cm height and 5 cm diameter) were collected from 16 plots (4 biochar rates x 4 plots, one sample per plot). Setting of plots was completely randomized among all plots of the field trial. Since the biochar was incorporated into the upper 15 cm layer, soil samples were collected in the centre (5-10 cm) and just below (15-20 cm) this layer to account for a possible downward movement of biochar. Here we only report on the 5-10 cm layer where the significant effects of biochar were predominant. Samples were collected in a moist soil between rows of rice in February 1, 2011 and February 15, 2012. The soil WRC was determined according to Embrapa (1997), adapted from Freitas Jr. and Silva (1984) and Reatto et al. (2008). Samples were saturated with water for 12 h and analysed in a Kokusan H-1400pF®centrifuge, four samples at a time, for 30 minutes under seven speed levels: 600, 700, 800, 1300, 1800, 2400 and 9100 rpm, corresponding to 0, 33.00, 44.92, 58.67, 154.93, 297.03 and 528.05 *g*. The volume of the soil water in the samples subjected to different speeds corresponded to seven matric potentials: -6, -8, -10, -33, -60, -100 and -1500 kPa. The bulk density was determined as the ratio between the dried mass of soil and the volume of the cylinder. Bulk density was used to calculate the volumetric soil moisture (cm^3 cm^{-3}). Saturated soil moisture was determined as the soil moisture content in saturated samples at 0 kPa right before subjecting samples to different speeds in the centrifuge. The relation between observed volumetric soil moisture and soil matric potential (the soil water retention curve - SWRC) and the predicted volumetric soil moisture ($\hat{\theta}$) was determined by fitting the nonlinear mixed (NLM) model described in Carvalho et al. (2014). Shape parameters of the SWRC were estimated using the maximum likelihood method, implemented in NLMIXED Procedure of the SAS/STAT® software (SAS Institute Inc., 2008). Comparisons of shape parameters between control and treatments with biochar were performed by *t*-tests for linear contrasts.

Considering the soil bulk density and depth where biochar was applied, the rate of biochar was equivalent in a dry mass basis to 0.4, 0.8 and 1.6% w/w. The response of soil physical hydric variables to biochar rate was evaluated via measurements of: i) soil bulk density (BD); ii) predicted soil moisture content ($\hat{\theta}$) at a matric potential *k* (k = 0, -6, -8, -10, -33, -60, -100 and -1500 kPa); iii) macro porosity (MAC) as the predicted soil moisture content between 0 and -6 kPa ($\hat{\theta}_0 - \hat{\theta}_6$); vi) rice stress free available water (RAW) as the predicted soil moisture content between -6 and -100 kPa ($\hat{\theta}_6 - \hat{\theta}_{100}$); and v) plant available water (PAW) as the predicted soil moisture content between -6 and -1500 kPa ($\hat{\theta}_6 - \hat{\theta}_{1500}$). For sensitivity analysis we also investigated biochar effects on RAW calculated with -100 kPa threshold, based on Wopereis et al. (1996) who showed that approximately below this matric potential drought stress effects on rice start to appear.

Response of physical hydric soil variables to biochar rates were analysed for each season separately via the quadratic model described in Equation 2:

$$y_{ij} = \beta_0 + \beta_1 char_i + \beta_2 char_i^2 + e_{ij} \qquad \text{(Eq. 2)},$$

where y_{ij} is the observation of the response variable y corresponding to biochar level i (i = 0, 8, 16, 32 t ha^{-1}) of the replication j (j = 1, 2, 3, 4); β_0 is the intercept; β_1 and β_2 are the linear and quadratic effects of biochar, respectively; and e_{ij} is the random error associated to each observation y_{ij}.

Analyses were performed using the MIXED procedure (Proc MIXED) of the statistical software SAS/STAT® (SAS Institute Inc., 2008). The magnitude of the biochar effect was assessed by nominal significance levels (p-values) derived from hypothesis testing of β_1 and β_2 estimates. Again, due to the large experimental area, relatively high residual variances were anticipated. For that reason, we adopted again 0.10 as the appropriate significance level for the selection of model predictors in order to safeguard against high type II error.

3. Results

3.1. The response of soil chemical properties to biochar and N application rates

Soil pH increased linearly ($p \leq 0.10$) with biochar rate at S1.5, but such a response was not observed at S2.5 and S3.5. In S3.5, the response of soil pH to biochar rates was quadratic with minimum at 16 t ha^{-1} (Table 2). Similarly, the Al availability decreased linearly with biochar application rate, but only at S1.5. Soil pH decreased, whereas Al availability increased with increasing N rate along all 3 seasons (Table 2). Amongst cations, there was a consistent positive effect of biochar amendment on K and Mg availability throughout the seasons. Potassium availability increased linearly, proportional to biochar rate (Table 2). The response of Mg to biochar was linear at S1.5, but quadratic at S2.5 and S3.5 with minimum around 16 t ha^{-1} (Table 2). No response of P availability to biochar application rates was observed (Table 2). The CEC corresponded to biochar rates in a quadratic manner with minimum around 8 t ha^{-1} at S1.5 and 16 t ha^{-1} at S2.5, whereas no effect of biochar on CEC was observed at S3.5. The SOM increased linearly with increasing biochar rates irrespective to N applied at S2.5 and S3.5 (Table 2, Fig. 3 a); and the evidence of the response of SOM to biochar rate was stronger at S3.5 ($p \leq 0.05$) than at S2.5 ($p \leq 0.10$) (Table 2).

Table 2. Response surfaces representing the effect of biochar (char) and N fertilisation (N) rates on soil chemical properties within 0-20 cm soil layer at 1.5 (S1.5), 2.5 (S2.5) and 3.5 (S3.5) years after application in a clay Rhodic Ferralsol.

Variable	Fitted model	R^2
	--S1.5--	
pH	$5.16 + 0.00394\ char^{*} - 0.00643\ N^{***}$	0.86
Ca	$10.48 - 0.1705\ char^{ns} - 0.06216\ N^{***} + 0.006887\ char^{2\ **}$	0.72
Mg	$3.38 + 0.01938\ char^{*} - 0.01526\ N^{***}$	0.62
Al	$3.54 - 0.03045\ char^{**} + 0.03419\ N^{***}$	0.88
K	$70.42 + 0.6971\ char^{***} - 0.4460\ N^{***}$	0.86
P	29.95	0.00
CEC	$19.25 - 0.176\ char^{ns} - 0.05468\ N^{***} + 0.00734\ char^{2\ **}$	0.66
SOM	$25.39 - 0.1338\ char^{ns} + 0.005224\ char^{2\ *}$	0.12
	--S2.5--------------------------------------	
pH	$5.10 - 0.00518\ N^{***}$	0.64
Ca	$8.32 - 0.04731\ N^{**}$	0.53
Mg	$3.82 - 0.05909\ char^{*} - 0.0586\ N^{**} + 0.000463\ N^{2\ *} + 0.002257\ char^{2\ **}$	0.67
Al	$3.16 + 0.04851\ N^{***}$	0.79
K	$94.03 + 0.5852\ char^{***} - 0.6318\ N^{***}$	0.86
P	$22.46 + 0.1144\ N^{**}$	0.25
CEC	$17.47 - 0.1438\ char^{ns} - 0.03155\ N^{*} + 0.005835\ char^{2\ *}$	0.50
SOM	$25.50 + 0.06684\ char^{*}$	0.16
	--S3.5--------------------------------------	
pH	$5.68 - 0.01127\ N^{***} - 0.01682\ char^{*} + 0.000532\ char^{2\ **}$	0.94
Ca	$11.16 - 0.09306\ N^{***}$	0.78
Mg	$4.30 - 0.07509\ char^{*} - 0.0325\ N^{***} + 0.002032\ char^{2\ *}$	0.86
Al	$0.55 + 0.06871\ N^{***}$	0.86
K	$68.55 + 1.4102\ char^{***} - 0.4161\ N^{***} - 0.01619\ char{\times}N^{**}$	0.89
P	24.72	0.00
CEC	$18.08 - 0.07675\ N^{***}$	0.53
SOM	$29.87 + 0.06896\ char^{**}$	0.37

pH (H_2O); Ca, Mg, Al ($mmol_c\ kg^{-1}$); K, P ($mg\ kg^{-1}$); CEC ($mmol_c\ kg^{-1}$); SOM ($g\ kg^{-1}$). Nominal significance level for *t*-tests of estimates: $^{***}p \leq 0.01$, $^{**}0.01 \leq p \leq 0.05$, $^{*}0.05 \leq p \leq 0.10$, ^{ns} not significant; and R^2: squared Pearson correlation coefficient between measured and estimated means (n = 16).

a) b)

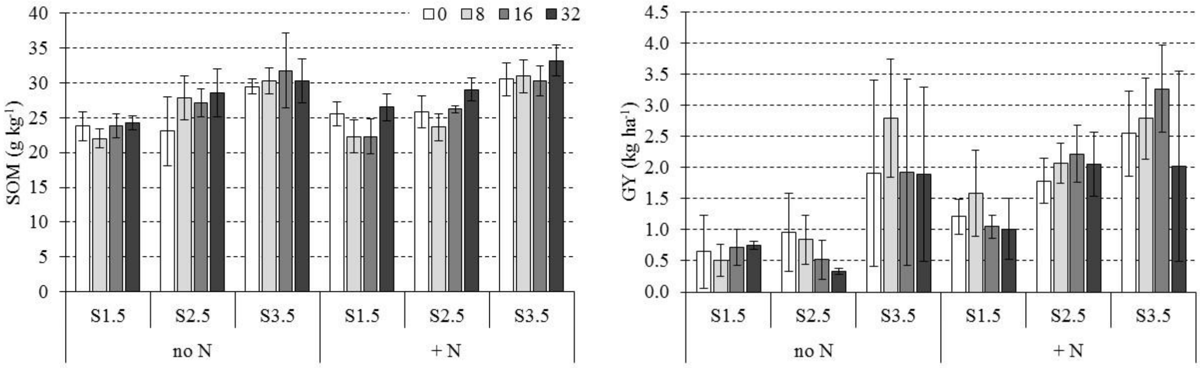

Figure 3. Observed response of soil organic matter (SOM - a) and rice grain yield (GY - b) to biochar rate (0, 8, 16 and 32 t ha^{-1}), without (no N) and with 90 kg N ha^{-1} (+N), at 1.5 (S1.5), 2.5 (S2.5) and 3.5 (S3.5) years after application on a clay Rhodic Ferralsol. Error bars represent the standard deviation of means (n = 4).

3.2. The response of SWRCs and soil physical hydric variables to biochar rate

Overall, summary goodness-of-fit measures (R^2: 0.89 to 0.98; RMSE: 0.017 to 0.007 cm^3 cm^{-3}) indicated that of the NLM model was adequate to estimate the shape parameters of the SWRCs (Fig. 4 c, d).The uncertainty was higher in S1.5 than in S2.5, probably due to the lack of more measured data points, especially at high matric potential, between saturated soil moisture and soil moisture content at -6 kPa. The shape parameters of the SWRCs were not significantly affected by biochar application rate (Table 3).

Table 3. Predicted shape parameters of the Van Genutchen model fitted to water retention data at 1.5 (S1.5) and 2.5 (S2.5) years after application of 8, 16 and 32 t ha^{-1} in a clay Rhodic Ferralsol.

Treatment	Parameters (S1.5)		R^2	Parameters (S2.5)		R^2
	-----------α--------	---------n----------		-----------α----------	---------n--------	
control	0.0298 (0.0088)	1.677 (0.070)	0.93	0.1040 (0.0302)	1.480 (0.033)	0.98
8	0.0227 (0.0067)	1.703 (0.072)	0.86	0.0741 (0.0200)	1.512 (0.032)	0.98
16	0.0194 (0.0070)	1.701 (0.088)	0.86	0.1624 (0.0509)	1.431 (0.032)	0.97
32	0.0284 (0.0096)	1.638 (0.076)	0.86	0.2144 (0.0709)	1.416 (0.033)	0.98

Standard error of estimates are between brackets; R^2: Pearson correlation coefficients between measured and predicted means of soil moisture content (n = 24). Shape parameters of the SWRCs with biochar were not significantly different from control (p > 0.10). Measurements taken within 5-10 cm soil layer.

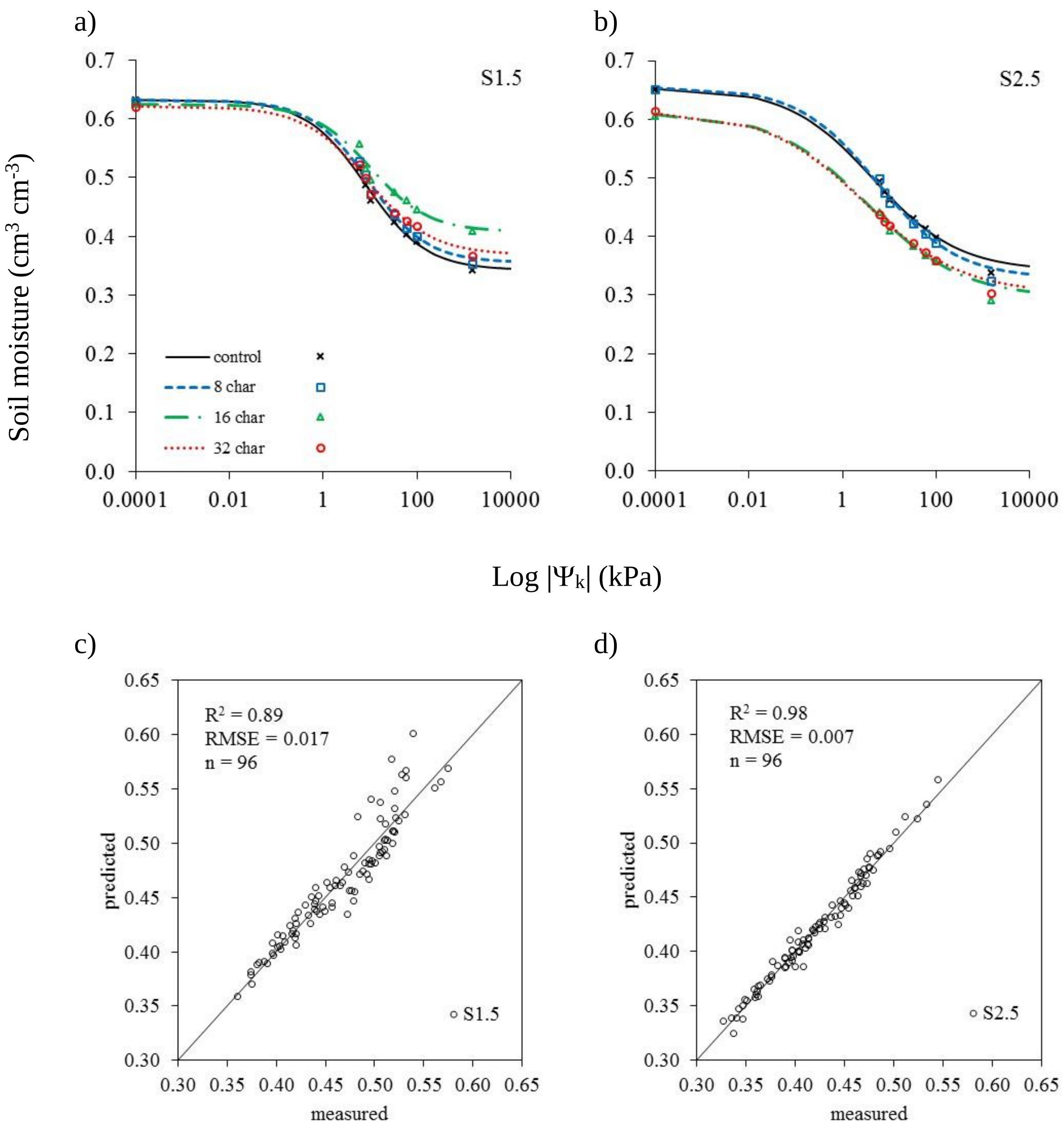

Figure 4. Predicted (lines) soil water retention curves and measured soil moisture (symbols) at a matric potential *k* (k = 0, -6, -8, -10, -33, -60, -100 and -1500 kPa) within 5-10 cm layer obtained at 1.5 (S1.5 - a) and 2.5 (S2.5 - b) years after application of biochar (8, 16 and 32 t ha^{-1}) in a clay Rhodic Ferralsol. Estimates of shape parameters are presented in Table 3. Goodness of fit of the nonlinear mixed model used to predict soil water retention capacity, summarized via correlation coefficient (R^2) and root of mean square error (RMSE) in S1.5 (c) and in S2.5 (d).

In both seasons, effects of biochar application rate on BD, soil moisture content at a matric potential between -6 and -60 kPa and RAW were consistently observed (Table 4). In S1.5, the response of BD and soil moisture content at a matric potential between -6 and -1500 kPa followed a quadratic pattern with maximum at 16 t ha^{-1}; accompanied also by a quadratic response of MAC with minimum at 16 t ha^{-1} and a linear decrease of RAW with biochar application rate (Table 4). The estimated BD (g cm^{-3}) was 1.31, 1.35, 1.36 and 1.32 with 0, 8, 16 and 32 t ha^{-1} biochar, respectively. Amongst soil physical hydric variables, the most evident effect of biochar was on MAC ($p \leq 0.01$). The estimated MAC (cm^3 cm^{-3}) was 0.1337, 0.1080, 0.0955 and 0.1098 with 0, 8, 16 and 32 t ha^{-1} biochar, respectively.

One year later, in S2.5, BD and soil moisture content at a matric potential between 0 and -60 kPa decreased linearly with biochar application rate; accompanied by a linear decrease of RAW with biochar amendment (Table 4). The evidence of the effect of biochar ($p \leq 0.01$) was stronger for BD, soil moisture content at matric potential -6, -8 and -10 kPa, and RAW. However, no effect of biochar was observed on soil moisture content at -100 and -1500 kPa matric potentials. The uncertainty of the response of RAW to biochar rate was higher in S1.5 ($p \leq 0.10$) than in S2.5 ($p \leq 0.05$) (Table 4). The RAW decreased consistently with about 0.6% for each t ha^{-1} of biochar amendment in both seasons. The response of PAW to biochar application rate was not statistically significant neither in S1.5 ($p = 0.1396$) nor in S2.5 ($p = 0.1724$) (Table 4).

Table 4. Response of physical hydric soil variables to biochar rate (char) at 1.5 (S1.5) and 2.5 (S2.5) years after application in a clay Rhodic Ferralsol.

Variable	Fitted model	R^2	Fitted model	R^2
	---------------------------S1.5------------------------		-----------------------S2.5---------------------	
BD	1.3124 +0.0061char * -0.0002 char$^{2\ *}$	0.88	1.3085 -0.0030 char **	0.64
$\hat{\theta}_0$	0.6329	0.00	0.6539 -0.0014 char *	0.67
$\hat{\theta}_6$	0.4996 +0.0035 char * -0.0001 char$^{2\ *}$	0.85	0.4884 -0.0019 char **	0.71
$\hat{\theta}_8$	0.4852 +0.0038 char * -0.0001 char$^{2\ *}$	0.83	0.4771 -0.0018 char **	0.71
$\hat{\theta}_{10}$	0.4739 +0.0040 char * -0.0001 char$^{2\ *}$	0.82	0.4685 -0.0017 char **	0.71
$\hat{\theta}_{33}$	0.4184 +0.0048 char * -0.0001 char$^{2\ *}$	0.77	0.4259 -0.0015 char *	0.71
$\hat{\theta}_{60}$	0.3964 +0.0051 char * -0.0001 char$^{2\ *}$	0.75	0.4079 -0.0013 char *	0.69
$\hat{\theta}_{100}$	0.3813 +0.0053 char * -0.0001 char$^{2\ *}$	0.75	0.3946	0.00
$\hat{\theta}_{1500}$	0.3354 +0.0062 char * -0.0002 char$^{2\ *}$	0.73	0.3351	0.00
MAC	0.1337 -0.0040 char *** +0.0001 char$^{2\ ***}$	0.84	0.1640	0.00
RAW	0.1140 -0.0007 char *	0.54	0.0938 -0.0006 char **	0.54
PAW	0.1566	0.00	0.1533	0.00

Data collected within 5-10 cm soil layer. Rate of biochar (0, 8, 16 and 32 t ha^{-1}). Soil bulk density (BD, g cm^{-3}), saturated soil moisture (θ_s), residual soil moisture (θ_r), macro porosity (MAC: $\hat{\theta}_0 - \hat{\theta}_6$), rice available water (RAW: $\hat{\theta}_6 - \hat{\theta}_{100}$) and plant available water (PAW: $\hat{\theta}_6 - \hat{\theta}_{1500}$). ($\hat{\theta}_k$) correspond to the soil moisture content (cm^3 cm^{-3}) at matric potential k, estimated via nonlinear modeling of soil water retention curves (Fig. 4). Nominal significance level of *t*-tests for the biochar effect: *** $p \leq 0.01$, ** $p \leq 0.05$, * $p \leq 0.10$. R^2: the squared Pearson correlation coefficient between measured and estimated means (n = 4).

3.3. Plant response to biochar and N application

In S1.5, total shoot dry matter (TDM) and grain yield (GY) decreased linearly ($p \leq 0.10$) with increasing biochar rate, but increased linearly with increasing N rate; the harvest index (HI) followed a quadratic response pattern to biochar application rates with maximum around 16 t ha^{-1} (Table 5). Estimated TDM and GY (mean ± standard error) varied from 2.03 ± 0.40 and 0.57 ± 0.20 t ha^{-1}, respectively, in treatments without N fertilization and with 32 t ha^{-1} to 3.89 ± 0.40 and 1.24 ± 0.20 t ha^{-1}, respectively, in treatments with 90 kg N ha^{-1} without biochar. In addition, the number of grains per panicle (GP, $p \leq 0.10$), 1000-grain weight (GW, $p \leq 0.10$) and concentration of N in grains (NG, $p \leq 0.05$) decreased linearly with biochar rate (Table 5). The application of N had generally a positive effect on GY and yield components, except for the number of healthy panicles (HP), which decreased linearly ($p \leq 0.01$) with increasing N rate (Table 5). The response of HP to biochar rate followed a quadratic pattern with maximum at 16 t ha^{-1}.

A year later, in S2.5, a significant interaction effect of biochar and N was observed on GY ($p \leq 0.01$), HI ($p \leq 0.10$) and NG ($p \leq 0.10$). The GY, HI and NG increased linearly with biochar rate if sufficient N fertilization was applied (rate > 60 kg ha^{-1}). In contrast, GY, HI and NG decreased linearly with biochar rate if no N fertilization was applied (Table 5). There was no effect of biochar on TDM, which increased linearly ($p \leq 0.01$) with N rate. Estimated GY and HI varied from 0.18 ± 0.20 and 0.25 ± 0.04, respectively, in treatments without N fertilization with 32 t ha^{-1} biochar to 2.04 ± 0.20 and 0.39 ± 0.04, respectively, in treatments with 90 kg N ha^{-1} and 32 t ha^{-1} biochar. The GW decreased linearly with biochar rate ($p \leq 0.10$) but increased with N rate. The HP was not affected by biochar rate, but decreased with N rate higher than 30 kg ha^{-1} (Table 5). Finally, in S3.5, no effect of biochar was observed on GY, which increased linearly ($p \leq 0.10$) with N rate (Table 5). The estimated GY varied from 2.13 ± 0.37 without N to 2.99 ± 0.36 with 90 kg N ha^{-1}, irrespective of biochar rate. The response of TDM and number of panicles m^{-2} (PAN) to biochar rate followed a quadratic pattern with maximum at 16 t ha^{-1}. The GP increased linearly ($p \leq 0.05$) with N rate, whereas the GW ($p \leq 0.10$) and HP ($p \leq 0.05$) decreased linearly with N rate. The HP increased linearly ($p \leq 0.05$) with biochar application rate (Table 5). The NG was not determined in S3.5. In S1.5 and S2.5 the GY was rather low (Fig. 3 b), mainly due to rice blast infestation. Although chemical control was applied when necessary, this could not sufficiently compensate for the low resistance of the cultivar BRS Primavera to rice blast (Pinheiro et al., 2006).

Table 5. Response surfaces representing the effect of biochar (char) and N-fertilisation rates on total shoot dry matter (TDM, t ha^{-1}), grain yield (GY, t ha^{-1}), harvest index (HI), and yield components of aerobic rice at 1.5 (S1.5), 2.5 (S2.5) and 3.5 (S3.5) years after application in a clay Ferralsol.

Variable	Fitted model	R^2
	-----S1.5-----	
TDM	2.59 -0.01739 char * +0.01451 N ***	0.70
GY	0.78 -0.00668 char * +0.00512 N **	0.56
HI	0.28 +0.005016 char * -0.00016 char$^{2\ *}$	0.29
PAN	143 +0.8082 N ***	0.67
GP	75 -0.4898 char * +0.2438 N *	0.76
GFI	0.40 +0.001897 N ***	0.36
GW	20.36 -0.05927 char *	0.24
HP	0.83 +0.00824 char * -0.0045 N *** -0.0003 char$^{2\ **}$	0.81
NG	1.13 -0.01243 char ** +0.0123 N ***	0.75
	-----S2.5-----	
TDM	1.86 +0.03643 N ***	0.91
GY	0.73 -0.01733 char ** +0.0099 N *** +0.00034 char×N ***	0.86
HI	0.33 -0.00251 char ^{ns} -0.00011 N ^{ns} +0.000053 char×N *	0.48
PAN	149 +1.4045 N ***	0.92
GP	96	0.00
GFI	0.60	0.00
GW	21.85 -0.03484 char * +0.01622 N *	0.39
HP	0.89 -0.0004 N ^{ns} -0.00005 N$^{2\ *}$	0.90
NG	1.27 +0.0352 char ^{ns} +0.01647 N *** +0.000505 char×N * -0.00192 char$^{2\ *}$	0.98
	-----S3.5-----	
TDM	3.00 +0.05942 char ^{ns} +0.02611 N *** -0.0023 char$^{2\ *}$	0.81
GY	2.13 +0.0096 N *	0.12
HI	0.37	0.00
PAN	213 +3.73374 char ** +1.04501 N *** -0.1161 char$^{2\ **}$	0.71
GP	106 +0.12973 N **	0.24
GFI	0.70	0.00
GW	27.81 -0.01444 N *	0.06
HP	0.81 +0.002732 char ** -0.00158 N **	0.59
NG	nd	nd

Rates of biochar (0, 8, 16 and 32 t ha^{-1}) and rate of N-fertilisation (0, 30, 60 and 90 kg ha^{-1}). PAN: number of panicles m^{-2}; GP: number of grains per panicle; GFI: grain filling index; GW: 1000-grain weight (g); HP: healthy panicles x 100 (%); NG: concentration of N in grains (g m^{-2}); nd: not determined. Nominal significance level for *t*-tests of estimates: $^{***}p \leq 0.01$, $^{**}0.01 \leq p \leq 0.05$, $^{*}0.05 \leq p \leq 0.10$, ns: not significant; R^2: squared Pearson correlation coefficient between measured and estimated means (n = 16).

4. Discussion

The main findings of this study were: i) biochar application enhances SOM at 2.5 and 3.5 years after application; ii) RAW decreases with biochar application rate at 1.5 and 2.5 years after application; and iii) the response of rice grain yield depends on changes in chemical and physical soil properties induced by biochar amendment with years after application.

In our field trial, millet was included in the crop rotation between the main crop, which can provide surplus of below and above ground biomass and favour an increment in SOM, especially under a no-tillage system (Six et al., 2004; Nascente et al., 2013a; Nascente et al., 2013b). This is evident from an overall relative increase in SOM of ~ 5 g kg^{-1} (or 0.5%) after 3.5 years of establishment of the field trial (Fig. 3 a). With addition of biochar, however, the SOM increased by 0.26% and 0.23% for each t ha^{-1} of biochar amendment in S2.5 and S3.5, respectively (Table 2). According to Zimmerman et al. (2011), SOM sorption to biochar can occur, with time, either onto external biochar surfaces or within biochar pores. The biochar used in our study has properties, such as the presence of functional groups (Table 1) and porosity (Fig. 1), that can favour both mechanisms. Besides, the magnitude of the increase in K availability with biochar rate was greater in S3.5 (1.4) than in S2.5 (0.6) and S1.5 (0.7) (Table 2). The effect of biochar on K availability with time (in S3.5) is certainly a consequence of the increase in SOM and soil pH rather than the inherently high K content in the biochar (Table 1) or the K applied at sowing every growing season. Soil pH increased by 0.11% for each t ha^{-1} of biochar applied at 0.5 year after application (Carvalho et al., 2013) and by 0.08% for each t ha^{-1} of biochar applied at 1.5 years after application (Table 2). The increase in soil pH is the most reported short-term effect on soil chemical properties with biochar amendment (Glaser et al., 2002; Lehmann et al., 2003; Steiner et al., 2007; Asai et al., 2009; Haefele et al., 2011). For aerobic rice, however, an increase in soil pH is probably not an advantage, since it can cause a decrease in crop uptake of important micronutrients, such as Zn, at early stages of rice growth (Fageria, 2000; Kreye, 2009). Yet, N biological fixing crops might benefit more of an increase in soil pH with biochar application (Rondon et al., 2007).

Contrary to the positive effects of biochar application on soil chemical properties, we found a constant decrease in RAW of 12% with 1% w/w in the upper 5-10 cm layer of the clay soil at 1.5 and 2.5 years after application (Table 4). Likewise, Tryon (1948) reported a decrease in both soil moisture content at a matric potential of approximately -100 kPa and wilting point that resulted in a reduction of available soil moisture content of 0.44% with 1%

(by volume) of wood biochar application under artificially controlled conditions. In our field trial, causes for a reduction in RAW seem to change with seasons. In S1.5, the main cause for a decrease in RAW is related to an increase in soil moisture content at -100 kPa rather than a decrease at -6 kPa (Fig. 4 a). The increase in soil moisture content at -100 to -1500 kPa matric potential can be a result of the micro porosity of the biochar used (Fig. 1). Yet, the contrasting effects of biochar application rate on BD and MAC point out that there were changes in the whole soil structure after application of biochar. Primarily, ploughing operations to incorporate biochar in the upper 0-15 cm soil layer leads to destruction of soil aggregates, which play an important role on water flow and retention capacity of a clay Ferralsol (Six et al., 2004). Secondly, a gradual compaction of the soil occurs due to sowing, fertilization, pulverization and weeding operations. If biochar adds an extra SSA of 32.4 m^2 g^{-1} (Table 1) to the clay soil and clay particles (< 2 μm) can fill biochar pore space (Fig. 1 c, d), then the higher the biochar rate the higher the relation mass/volume. However, the time required for filling biochar pore space is likely to be dependent on the rate of biochar. This is revealed by the lowered impact of biochar on BD and MAC at a rate > 16 t ha^{-1} (Table 4). A season later, in S2.5, BD decreased, whereas SOM increased with biochar rate. If SOM increases with biochar rate, then a decrease in BD is expected, primarily because SOM has in nature a very low BD (~ 0.3 g cm^{-3}). Further, the biochar-SOM-clay interaction might result in formation of new aggregates that can increase pore space in soil, decreasing BD. The decrease in BD with biochar application was reported in other studies on fine texture soils (Laird et al., 2010; Vaccari et al., 2011; Ventura et al., 2012; Major et al. 2012), although negative effects on soil WRC were not observed. The effect on BD is usually related to the inherent low bulk density of the biochar; however, along seasons in a cropping system, there might be different causes as observed in this study. Our results show that both saturated soil moisture and soil moisture content at -6, -8 and -10 kPa decreased with increasing biochar amendment, indicating that there were changes in soil porosity and structure within 2.5 years after biochar application leading to a decrease in WRC of the clay soil.

A decrease in RAW can have a relevant impact on rice performance under aerobic conditions in the Brazilian Savannah, especially during reproductive stage, between 45 and 75 DAE. The intentionally little supplemental irrigation applied (31 mm in S1.5 and 9 mm in S2.5, Fig. 2) was not sufficient to avoid water stress throughout the growing seasons. Water stress and a consequent decrease in N uptake can have a severe impact on aerobic rice yield via reduction of total shoot dry matter, number of panicles, grains per panicle, number of filled grains and weight of grain (Stone and Moreira, 1996; Stone et al., 1999; Fageria, 2001). In S1.5, we found a GY decrease with 0.8% for each t ha^{-1} of biochar applied or around 13% with 16 t ha^{-1}, irrespective of the level of N applied (Table 5). Similarly, Asai et al. (2009) observed a reduction of 27% in rice GY with 16 t ha^{-1} wood biochar and without N fertilization on a 48% clay soil, although no significant effects on soil WRC were reported. They attributed the reduction in GY to a decrease in N uptake due to possible N immobilization. In our field trial, a deficit in N uptake by the crop is likely due to water stress, considering that the effect of biochar on GY was irrespective of the level of N applied and the total amount of water supplied along the season. The total amount of water supplied during the reproductive stage of rice in January/February in S1.5 (500 mm) was about 24% lower than in S2.5 (658 mm) and 14% lower than in S3.5 (581 mm) (Fig. 2). Therefore, the weather condition in S1.5 was favourable for a more severe negative impact on GY due to a reduction in RAW with biochar application. This is evident from a reduction in TDM, GP and NG by 0.66%, 0.65% and 1.08% for each t ha^{-1} of biochar applied, respectively, or of around 11%, 10% and 17% with 16 t ha^{-1} biochar, respectively (Table 5). The water stress was also expressed by a reduction of 10% in GW with 32 t ha^{-1} biochar amendment and on the number of HP, which decreased with biochar rate > 16 t ha^{-1} (Table 5). The magnitude of the reduction in GW with biochar amendment was twofold greater in S1.5 than in S2.5 (Table 5).

The frequency for an amount of rainfall in January/February at Capivara Farm greater than 500 mm in the latest 33 years (1981-2013) was 24% (Agritempo, 2014); therefore, the S2.5 and S3.5 were relatively very wet seasons. Consequently, N availability is most likely the main factor related to the effect of biochar on rice performance in S2.5. This is confirmed by the significant interaction effect biochar × N on GY in S2.5 (Table 5). The GY in the treatment with 32 t ha^{-1} biochar only was ~ 82% lower than that of the treatment with maximum N-fertilizer rate (Fig. 3 b). On the other hand, 32 t ha^{-1} biochar ensured a 13% increase in rice GY under the highest N fertilization treatment relative to the highest N fertilizer treatment alone (Fig. 3 b). This relative increase in GY is higher than the 6% observed by Haefele et al. (2011) on a rain fed humic Nitosol at the third season of rice after application of 41 t ha^{-1} rice husk biochar with 'medium fertilizer rate'. On the other hand, this is considerably lower than the 73% found by Steiner et al. (2007) on a recently cleared area of forest on an Amazon Ferralsol with aerobic rice cultivated just after application of 11 t ha^{-1} wood biochar combined with N-P- K (85-75-100 kg ha^{-1}) and liming. A difference in history of soil cultivation is probably the main cause for the varying results. In our study, improvement of SOM with biochar application is likely the key reason for the interaction effect of biochar × N at 2.5 years after application. Soil organic matter can enhance retention of positively charged ions in soil, such as ammonium, benefiting N recovery by the crop from the mineral N fertilization (Fageria, 2012). In addition, the increase in SOM with biochar amendment can stimulate the activity of macro and microorganisms and possibly favour mineralization of biochar. If biochar is mineralized, then N immobilization by microorganisms increase and supplemental N is required to compensate the deficit in N availability to the crop. The negative effect biochar on GW and NG (Table 5) indicates a deficit in N availability to the crop in S2.5.

Finally, at 3.5 years after application, no response of rice GY to biochar was observed, even though TDM and PAN decreased with biochar application rate > 16 t ha^{-1} and HP increased by 0.34% for each t ha^{-1} of biochar amendment (Table 5). It is conceivable that the contrasting effect of biochar on PAN and HP cancelled each other out resulting in a lack of GY response. A positive effect of biochar on HP was also observed during the first growing season of this same experiment (Carvalho et al., 2013); a promising result, given that rice blast is an endemic disease that has a huge impact on aerobic rice grain yield in Brazil (Breseghello et al., 2011).

5. Conclusions

Biochar application affects many physical and chemical soil variables. The most prominent and persisting effects are (1) increased SOM and (2) decreased stress free available water for rice. Aerobic rice grain yield and yield components were a good indicator of the changes that biochar induced on soil chemical and physical properties over time. Rice grain yield varied with the seasons according to changes in soil properties and to the weather conditions. At 1.5 years after biochar application, grain yield decreased, irrespective of the level of N applied; at 2.5 years after application, grain yield increased with biochar amendment if sufficient N fertilization was applied; and at 3.5 years after application, the effect of biochar on grain yield was absent. Overall, biochar had either no effect or a negative effect on rice yields, biomass and/or yield components. Therefore, a single application of biochar rate up to 32 t ha^{-1} is insufficient to ensure aerobic rice yield increase and stability on a clay Ferralsol within 3.5 years after biochar application under the current drought prone environment. There are some indications that biochar applications might mitigate the occurrence of rice blast. Most likely, positive effects of soil chemical properties on rice yield were offset by negative effects of reduced soil water retention capacity and N uptake by the crop. Our results also suggest that other crops may benefit more because: i) no significant effect of biochar on total plant available water was observed, and ii) biochar with neutral pH and high ash, Ca and Mg contents, such as the one used in this study, triggers similar effects as that of liming. A more realistic assessment of biochar as a soil amendment that can increase crop production must come from longer-term field trials and should include the chemical, physical and biological components of soil fertility.

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Chapter 5

No effect of biochar amendment on N_2O-N fluxes along four cropping seasons on a clay Ferralsol

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"But science is not an Olympic sport, where faster, higher and longer are the only results that count."

(H. Jungkunst, Nature Geoscience, 2010)

Abstract

Nitrogen is an element involved in key process in soil and plant. The rational use of this resource is an important step towards smarter farming systems. In this context, the use of biochar is regarded as a soil amendment capable of reducing N losses via N_2O-N fluxes. However, consistent information about the impact of biochar on N_2O-N fluxes and the mechanisms involved in soil born N_2O-N fluxes with biochar amendment under real farming conditions are missing. Aiming to quantify the effect of a single application of wood biochar (32 Mg ha^{-1}) on N_2O-N fluxes, we used manual static chambers during four cropping seasons following single biochar application on a clay soil in the Brazilian Savannah. Soil variables related to N_2O-N fluxes, ammonium (N-NH_4^+) and nitrate (N-NO_3^-) and water filled pore space (WFPS), were measured alongside N_2O-N fluxes. Soil pH, soil organic matter (SOM) and grain yield were measured annually to ascertain any possible gradual effects of biochar. There were no effects of biochar amendment on N_2O-N fluxes along all seasons monitored. This observation was made both in plots without and in plots with annual N fertilization (90 kg N/ha/season). In the first season after application, biochar increased soil pH. In this season an interaction between biochar and N-application was observed for SOM, but at 2.5 years after application an increase in SOM with biochar was found irrespective of N application. At 1.5 and 2.5 years after application, biochar caused a decrease in WFPS. Concurrently, it was observed that in seasons characterized by low WFPS, fluxes of N_2O-N and soil N-NO_3^- and N-NH_4^+ availability were enhanced due to N fertilization. Our findings highlight the importance of longer-term field studies investigating and monitoring the impact of biochar on N_2O-N fluxes, and the dependency of the magnitude of N_2O-N fluxes on soil properties and irrigation regimes.

Keywords: nitrous oxide, wood biochar, mineral N fertilization, soil properties

1. Introduction

Biochar is the charred by-product of biomass pyrolysis (Sohi et al. 2010). A wood biochar is generally alkaline and rich in micro pores, characteristics that in theory would contribute to lower N losses via fluxes of nitrous oxide (N_2O-N) (Clough and Condron 2010). The N_2O is a potent greenhouse gas (GHG) with a global warming potential nearly 310 times higher than carbon dioxide (CO_2). It is an important component of gas emission coming from agricultural lands which account for around 14% of the total anthropogenic CO_2-eq emitted globally (IPCC 2007) and for 12.95% of the total emissions of Brazil (MCTI 2013). The N_2O emitted from agricultural fields is primarily a direct consequence of mineral and organic fertilization. In Brazil, agricultural fields are the source of 88% of the total anthropogenic N_2O (MCTI 2013). Potentially, biochar could contribute to mitigate GHG emissions through carbon sequestration and reduced N_2O emissions (Lehmann et al. 2006, Woolf et al. 2010). A number of studies have shown that biochar can reduce N_2O emissions (Lehmann et al. 2006, Sohi et al. 2010, Atkinson 2010, Cayuela et al. 2010, Spokas et al. 2009, Zhang et al. 2010), whereas others showed no effect (Scheer et al. 2011, Karhu et al. 2011). A recent meta-analysis by Cayuela et al. (2013) showed that biochar reduces soil N_2O emissions by on average 54%. However, of the 30 studies analysed only five were from field trials (Scheer et al. 2011, Taghizadeh-Toosi et al. 2011, Liu et al. 2012b, Zhang et al. 2012a, Zhang et al. 2012b). In short-term studies under pasture conditions, Scheer et al. (2011) found no effect and Taghizadeh-Toosi et al. (2011) found a positive effect of biochar on decreasing N_2O-N fluxes. In a two-year field trial under a Chinese rice paddy system, Liu et al. (2012b) and Zhang et al. (2012a) found a decrease in N_2O-N fluxes if N fertilization was combined with biochar amendment. Conversely, in a two year field trial Verhoeven and Six (2014) found no effect of biochar amendment in reducing N_2O-N fluxes from a commercial wine grape vineyard. Clearly, the effect of biochar and its combination with nitrogen application on N_2O emissions in field studies is the result of complex interactions and needs to be more thoroughly explored.

Fluxes of N_2O-N from soil are primarily dependent on soil properties, climate and fertiliser management. Biochar application and tillage operations affect soil properties such as pH, moisture and organic matter content, as well as soil structure, which in turn are, along with N fertilization, the main drivers of N_2O emission (Bouwman 1990). Some biochar effects on soil properties are short term, others only manifest themselves after longer time periods, depending on the interactions of biochar with soil matrix, a process known as "aging" (Kookana et al. 2011). The long-term interaction of biochar with soil matrix cannot be captured in laboratory studies. Mukherjee and Lal (2014) reported that contradictory results from laboratory versus field observations exist, calling for more careful extrapolation from laboratory studies to field conditions. Of particular relevance for biochar and N_2O emission are temporal dynamics whereby initially high emissions may decrease over time. The over-presence of laboratory studies and the lack of long term field studies on biochar effects on N_2O fluxes is therefore a problem.

Cayuela et al. (2013) also showed that reductions of N_2O emission are directly proportional to the amount of biochar applied (weight of biochar per weight of soil). Very often, the amount of biochar applied in laboratory studies is much higher than what is feasible under field conditions. For example, in an incubation experiment, Spokas et al. (2010) only found a significant decrease in N_2O emission with biochar amendment rate (w/w) higher than 20%. Such an application rate is improbable under field conditions. There is therefore a need for field studies of biochar effects on N_2O emission with realistic biochar rates. The amount of biochar applied in this study, namely 32 Mg ha^{-1} or 1.6% (w/w) is already high for field applications but still deemed feasible. We studied effects of a single application of wood biochar on N_2O emission over the course of four cropping seasons under ordinary agronomic management with seasonal N fertilization in the Brazilian Savannah.

Nitrogen is one of the most important nutrients for crop production. It is involved in a series of key processes in plant and soil, such as photosynthesis and maintenance of soil organic matter. In soil, there are two biochemical steps involved in N availability: nitrification and denitrification. Nitrification is the biological oxidation of ammonium ($N\text{-}NH_4^+$) into nitrite ($N\text{-}NO_2^-$) or nitrate ($N\text{-}NO_3^-$); and denitrification is the reduction of $N\text{-}NO_3^-$ into N_2 and nitrous oxide ($N_2O\text{-}N$) (Bouwman 1990, Davidson et al. 2000). Production of $N\text{-}NO_3^-$ is, therefore, dependent on $N\text{-}NH_4^+$ availability. The $N\text{-}NH_4^+$ available in soil is a result of mineralization of NH_3 present in soil organic matter or applied via mineral fertilizer, and part of it is used by microbes to grow and reproduce (immobilization). However, if there is a surplus of $N\text{-}NH_4^+$ in soil, then most probably there will be a surplus of $N\text{-}NO_3^-$ due to enhanced nitrification process resulting in production of $N_2O\text{-}N$, especially under aerobic conditions (Bremner 1997). Both $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ are available forms for plant uptake. If not used by plants, the extra $N\text{-}NO_3^-$ produced can be leached or reduced into N_2O, particularly under intermittent aerobic/anaerobic condition. Under continuously anaerobic conditions, $N\text{-}NO_3^-$ is likely to be reduced to N_2. Possibly, the changes in soil properties induced by biochar amendment, such as increased soil source of C, can favour N_2O-N losses via nitrification, which, according to Parton et al. (1988), is a typical pathway under aerobic conditions with high availability of $N\text{-}NH_4^+$. Applications of excessive amounts of biochar and mineral N to soils provide the necessary C and $N\text{-}NH_4^+$ for nitrifying bacteria to produce $N\text{-}NO_3^-$. Further, biochar can change soil water dynamics, affecting the frequency and duration of aerobic soil conditions. Changes in soil N availability and water dynamics caused by biochar can affect N_2O emission and crop growth. Crop growth in turn also affects water dynamics and soil N availability for the bacteria responsible for N_2O production. Therefore, N_2O-N fluxes are important indicators of the impact that biochar can have in the entire cropping system. In this study we tested a by-product of charcoal production from plantation timber as a soil amendment in a cropping system. This biochar is potentially available in large quantities in the Brazilian savannahs, but the value for agriculture is yet unclear.

Previously, we reported positive effects of biochar on soil chemical properties, but negative effects on soil water retention capacity in the same field trial where this study was conducted. The period of observation covered 0.5 to 3.5 years post biochar application (Carvalho et al. 2013b, Carvalho et al. under review). Here we analyse effects of biochar on N_2O-N fluxes. Our objective was to investigate the impact of a single application of wood biochar, combined with annual N fertilization, on N_2O-N fluxes from immediately up to 2.5 years after biochar application. We established a field trial involving strategic key staple food crops on a clay Ferralsol in the Brazilian savannah. The cropping system was conducted under zero-tillage with rotation including common bean, millet and aerobic rice. We assessed the impact of wood biochar without or with mineral N fertilization on N_2O-N fluxes, and related soil variables, throughout four cropping seasons. We tested the following hypotheses: i) initially, the high rate of labile C added to the soil with biochar application leads to a decrease in mineral N availability and lowered N_2O-N fluxes; ii) with time, biochar amendment increases the capacity of soil to retain mineral N, lowering N_2O-N fluxes compared to application of mineral N alone; iii) increases in water filled pore space resulting from biochar application trigger intermittent aerobic/anaerobic states in the soil, thereby favouring N_2O-N fluxes.

2. Material and Methods

2.1. Set up of the field trial and design

The field trial was established in June 2009 on a clay Rhodic Ferralsol at Capivara farm of the Brazilian Research Centre for Rice and Beans (Embrapa Arroz e Feijão) in Santo Antônio de Goiás, Goiás State, Midwest Region of Brazil (16°29'17 "S and 49°17'57 "W). Initial chemical properties of the clay soil are shown in Table 1. The trial was conducted under centre pivot irrigation. Since 2001, the area had been cultivated under zero-tillage with an intercrop of corn (*Zea mays*) with grass (*Urochloa ruziziensis*) during wet season (November to March), followed by irrigated common bean (*Phaseolus vulgaris*) during dry season (June to August). Immediately after establishment of the field trial, irrigated common bean was cultivated as the first crop during dry season, followed by rice (*Oryza sativa*). This was

Table 1. Initial properties of the clay Ferralsol and characterization of the wood biochar used in the field trial.

Properties	Clay Soil	Biochar
Clay (g kg^{-1})	574	-
Sand (g kg^{-1})	326	-
Silt (g kg^{-1})	100	-
SOM (g kg^{-1})	20	-
Ca ($cmol_c$ kg^{-1})	1.6	2.5
Mg ($cmol_c$ kg^{-1})	0.6	0.8
Al ($cmol_c$ kg^{-1})	0.2	0
P (mg kg^{-1})	10	72
K (mg kg^{-1})	94	780
pH (H_2O)	5.1	7.6
pH ($CaCl_2$) 0.01M	-	6.4
C (% weight)	-	75.89
N (% weight)	-	0.78
Oxidisable C (% weight)	-	5.89
Carboxyl groups (meq g^{-1})	-	0.41
Lactone groups (meq g^{-1})	-	0.16
Phenol groups (meq g^{-1})	-	0.35
Ash content (meq g^{-1})	-	0.45
pZ at pH 4.8 (mV)	-	-34
SSA (m^2 g^{-1})	-	32.40

SOM: soil organic matter, SSA: specific surface area

repeated for subsequent cropping seasons, except that a third crop, millet (*Pennisetum glaucum*), was grown in 2011 between rice and common bean. At establishment of the field trial, the soil was ploughed twice to a depth of 0-20 cm in order to incorporate crop residues and guarantee a uniform incorporation of biochar into the soil.

The biochar was applied only once, in June 9, 2009. Chemical properties of biochar are presented in Table 1. Biochar was milled to pass through a 2000-μm sieve, spread manually over the soil surface, and incorporated to a depth of approximately 10-15 cm using a harrow. Four rates of biochar (0, 8, 16, 32 Mg ha^{-1}) were applied in randomly distributed design to plots of 40 m^2 (4 m × 10 m). Sixty four experimental plots of 40 m^2 were arranged in four blocks with combinations of biochar and mineral N fertilizer (0, 30, 60 and 90 kg ha^{-1}). Plots had to be placed out the track of pivot wheels resulting in one incomplete block. For operational reasons, the same N rate was applied in sequential strips across the four blocks every growing season. Here we report on the effect of the maximum biochar rate (32 Mg ha^{-1}) on plots without and with 90 kg N ha^{-1} during the first (16/06/09 to 21/09/09), second (03/11/09 to 22/02/10), third (08/11/10 to 21/02/11) and fourth (28/11/11 to 19/03/12) cropping seasons after biochar application. These seasons are equivalent to immediately (S0.0), 0.5 (S0.5), 1.5 (S1.5) and 2.5 (S2.5) years after biochar application to the clay soil. Every plot was given the same rate of P-K (kg ha^{-1}) at sowing: 15-20 in S0.0, 120-60 in S0.5, 60-30 in S1.5, and 30-30 in S2.5. Mineral N (urea) was divided into two or three applications. In S0.0, 5 kg N ha^{-1} was applied at sowing and 85 kg N ha^{-1} at 30 days after sowing (DAS). In S0.5, 45 kg N ha^{-1} was applied at sowing and 45 kg N ha^{-1} at 40 DAS. In S1.5, 45 kg N ha^{-1} was applied at sowing and 22.5 kg ha^{-1} at 30 and 50 DAS. Finally, in S2.5, 36 kg N ha^{-1} was applied at sowing and 27 kg N ha^{-1} at 30 and 50 DAS.

Monthly precipitation, irrigation and average maximum and minimum temperatures throughout cropping seasons are presented in Fig. 1, according to the weather station of Capivara farm and database of Agritempo (2014). Along the dry season, in S0.0, 10.2 mm was applied at each 3 days throughout the growing season from 17/06/09 to 09/09/09, resulting in a total amount of ~ 573 mm of water supplied via irrigation and rainfall (316 mm applied via irrigation and ~ 257 mm supplied via rainfall). During the wet season irrigation was applied only subsequently to more than six days of dry weather, in order to avoid crop failure. In S0.5, the amount of irrigation was 78 mm in amounts of 13 mm in January (at 2, 14 and 16 DAS) and February (at 89, 93 and 95 DAS), resulting in a total amount of ~ 966 mm supplied via irrigation and rainfall. In S1.5 the amount of irrigation was 9 mm in November (1 DAS), 23 mm in January (between 65 and 80 DAS), and 9 mm in February (between 91 at 97 DAS), resulting in a total amount of ~ 1040 mm supplied via irrigation and rainfall. Finally, in

S2.5 only 9 mm was applied in February (at 95 DAS), resulting in a total amount of ~ 1022 mm supplied via irrigation and rainfall over the season.

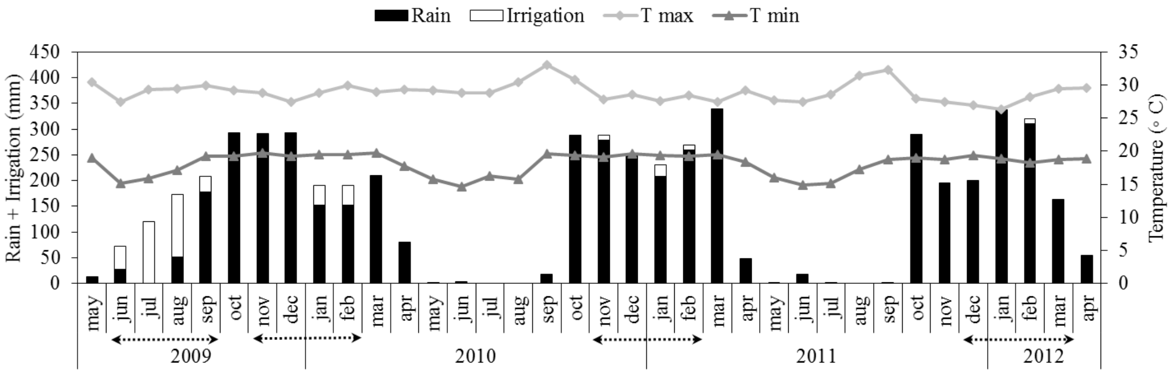

Figure 1. Monthly precipitation (Rain), irrigation and average maximum (T max) and minimum (T min) temperatures throughout cropping seasons. Arrows indicate whole period within cropping seasons when N_2O-N fluxes were measured.

2.2. Measurement of N_2O-N fluxes

Measurements were taken in 16 plots using manual static chambers. In S0.0 and S0.5 there was one static chamber per plot, and in S1.5 and S2.5 there were two static chambers per plot. The manual static chamber consisted of a metal base (0.38 m wide x 0.58 m long) covering a soil area of 0.22 m^2 and a plastic cap (of 0.1 m height) fixed on the metal base, similar to the chambers used by Carvalho et al. (2013a). In S2.5, the plastic covers were substituted by metal covers with same dimensions as described above. The cover was always protected with an insulation foil to keep temperature inside the chamber as stable as possible at the moment of sampling. When closed, the volume of the chamber was 19.8 L. Fluxes were measured weekly and 3 to 6 consecutive days after sowing and synthetic N fertilization events. Gas samples were taken between 9:00 and 11:00 a.m., as recommended by Alves et al. (2012). Gases accumulated in the static chamber in a period of 30 minutes were collected using manual vacuum pump. Following, gas samples were analyzed via gas chromatography with an Electron Capture Detector (ECD) (Auto system XL, Perkin Elmer) calibrated with certified N_2O standards of 350 and 1000 ppb (White Martins Gases Industrials Ltda.). The air temperature was measured simultaneously with N_2O-N flux sampling. Fluxes of N_2O-N (µg m^2 per hour) were calculated according to Rochette et al. (2004).

2.3. Measurement of soil related variables

Soil moisture, ammonium ($N\text{-}NH_4^+$) and nitrate ($N\text{-}NO_3^-$) concentrations were determined from 100 g soil samples within 0-10 cm soil depth simultaneously with N_2O-N sampling. Around 10 g of soil was weighed before and after drying in an oven for 24 hours in a temperature of 105 ºC. The water filled pore space (WFPS) was calculated by considering the soil moisture (g g^{-1}) at the moment of N_2O-N sampling, the soil bulk density (g cm^{-3}) and the mineral particle density (g cm^{-3}). Mineral particle density, 2.53 g cm^{-3}, was determined once prior to establishment of field trial. Soil bulk density was determined for each plot every growing season via undisturbed cores collected in a metal ring of known volume and calculated from the dry mass of soil per volume. The WFPS was calculated according to Paul & Clark (1996), cited by Giacomini et al. (2006). The available ammonium and nitrate were extracted from soil samples by shaking 20 g of soil with 60 mL of 1M KCl for 60 minutes according to Mulvaney (1996). Extraction was followed by determination via flow injection analysis (Ocean Optics, USA). The final result was given in mg L^{-1}. To estimate mineral N (mg kg^{-1}) the soil moisture at the moment of sampling was taken into account.

2.4. Measurement of soil pH, soil organic matter and grain yield

Soil pH and soil organic matter (SOM) were determined once, around 80 DAS within each cropping season. Three soil sub-samples of 500 g at 0-20 cm soil depth were collected from each plot (between rows of the crop). Soil samples were air dried and milled to pass through a 2000-µm sieve. The SOM was determined using the Walkley-Black method (Nelson and Sommers 1996) and the soil pH using a 1:1 soil:water solution (Bates 1973) adapted by Embrapa (2009). At crop maturity, around 90 DAS for common bean and 105 DAS for rice, grain yield (weight of grains dried to 13% moisture) was determined in samples collected from 2 rows of 3-m length in the centre of each plot.

2.5. Statistical analysis

The influence of N, biochar and its interaction on N_2O-N fluxes and soil related variables ($N\text{-}NH_4^+$, $N\text{-}NO_3^-$ and WFPS) was assessed via linear mixed modelling. Comparisons of averaged fluxes and soil related variables for the entire season (94 days after sowing - DAS - in S0.0; 107 DAS in S0.5; 106 DAS in S1.5; and 113 DAS in S2.5) and for pre-established periods within each season along 3 to 6 consecutive days after N fertilizations (FERT I: at sowing event, FERT II and FERT III: top dressings around flowering event) were done via F tests for contrasts. We included plot as random effect in order to account for correlations among measurements taken in the same plot along each cropping season and periods after N fertilization, which characterizes a repeated measures data set. Analysis was performed for each cropping season separately. The model for each season is described in Equation 1:

$$y_{ijk} = \mu + N_i + CHAR_j + N_i * CHAR_j + u_{ijk} + e_{ijk} \quad (1),$$

where μ is the overall mean; y_{ijk} is the observation of the response variable y corresponding to the i-th level of N fertilization, i = (0, 90 kg N ha^{-1}), and j-th level of biochar amendment, j = (0, 32 Mg ha^{-1}), of the replication k, k = (1, 2, 3, 4); N_i is the effect of the i-th N level and $CHAR_j$ is the effect of the j-th biochar level; N_i*$CHAR_j$ is the effect of the interaction between the i-th N level and the j-th biochar level; $u_{ijk} \sim N(0, \Sigma)$, is the random effect to account for potential correlations among repeated measurements taken within the same plot ijk (ijk = 1, …, 16); and $e_{ijk} \sim N(0, \sigma^2)$, is the random error associated to each observation y_{ij}.

Correlations between measured fluxes and soil related variables within each season were determined by the Pearson correlation coefficient (R^2). For soil pH, SOM and grain yield, which were variables measured annually, analysis was performed considering location of a plot as a random effect. The model is described in Equation 2:

$$y_{ijcr} = \mu + N_i + CHAR_j + N_i * CHAR_j + u_c + w_r + e_{ijcr} \quad (2),$$

where μ is the overall mean; y_{ijcr} is the observation of the response variable y corresponding to the i-th level of N fertilization, i = (0, 90 kg N ha^{-1}), and the j-th level of biochar amendment, j = (0, 32 Mg ha^{-1}), of the replication k (k = 1, 2, 3, 4); N_i is the effect of the i-th N level and $CHAR_j$ is the effect of the j-th biochar level; N_i*$CHAR_j$ is the effect of the interaction between the i-th N level and the j-th biochar level; u_c and $w_r \sim N(0, \Sigma)$, are the random effects to account for potential spatial effect related to localization of a plot in a column c (c = column 1, …, column 9) and in a row r (r = row 1, …, row 11); and and $e_{ijcr} \sim N(0, \sigma^2)$, is the random error associated to each observation y_{ij}.

Whenever F tests indicated significant effects ($\alpha \leq 0.05$) for the interaction N*CHAR, then sliced F tests for the effects of biochar within N treatments are presented. Otherwise, only F tests for the main effects N and CHAR are presented. Analyses were performed using the linear mixed model procedure (Proc MIXED) and the correlation procedure (Proc CORR) of the statistical software SAS/STAT® (SAS Institute Inc. 2008).

Uncertainties in estimation of total emission via integration of fluxes over time using measurements of manual static chambers can be as large as 50% (Kroon et al. 2010, Flechard et al. 2007). Therefore, we decided to present and compare daily and averaged fluxes of N_2O-N in the first instance before aggregating the data, which finally can be used as an estimate of total emissions over the entire growing seasons. To our knowledge, manual static chambers are still the most appropriate method to take direct measurements at plot scale.

3. Results

3.1 Patterns of daily N_2O-N fluxes along cropping seasons

Magnitude of daily N_2O-N fluxes were generally lower throughout S0.0 and S0.5 (Fig. 2 - a, b) than in S1.5 and S2.5 (Fig. 2 - c, d). Negative fluxes occurred in 36% and 38% of days measured in S0.0 and S0.5, respectively, but only in 22% and 23% of days measured in S1.5 and S2.5, respectively. A comparison of maximum fluxes within each treatment yielded the following results. In S0.0, highest maximum flux (µg m^{-2} per hour) occurred already at 1 day after sowing (DAS) in the treatment with N without biochar (21.18 ± 16.71). This was followed by the treatment with N and biochar (17.54 ± 6.23) and the treatment without N and biochar (13.02 ± 8.69) also at 1 DAS. The lowest of the maximum fluxes was observed in the treatment without N and with biochar (12.84 ± 9.47) at 30 DAS. In S0.5, highest maximum fluxes also occurred at the start of the season (1 DAS) in the treatment with N without biochar (23.80 ± 6.98), followed by the treatment without N and biochar (23.15 ± 21.21), with N and biochar (20.88 ± 15.32) and the treatment without N with biochar (11.32 ± 18.81). In S1.5, the highest maximum flux occurred in treatment with N and biochar (69.35 ± 37.80) at 8 DAS. This was followed by the treatment without N with biochar (61.74 ± 33.39) at 22 DAS, the treatment with N without biochar (48.00 ± 16.99) at 8 DAS, and the treatment without N and biochar (39.83 ± 23.06) at 2 DAS. Finally, in S2.5, maximum fluxes for all treatments occurred again at 1 DAS. Highest maximum flux was observed in the treatment with N

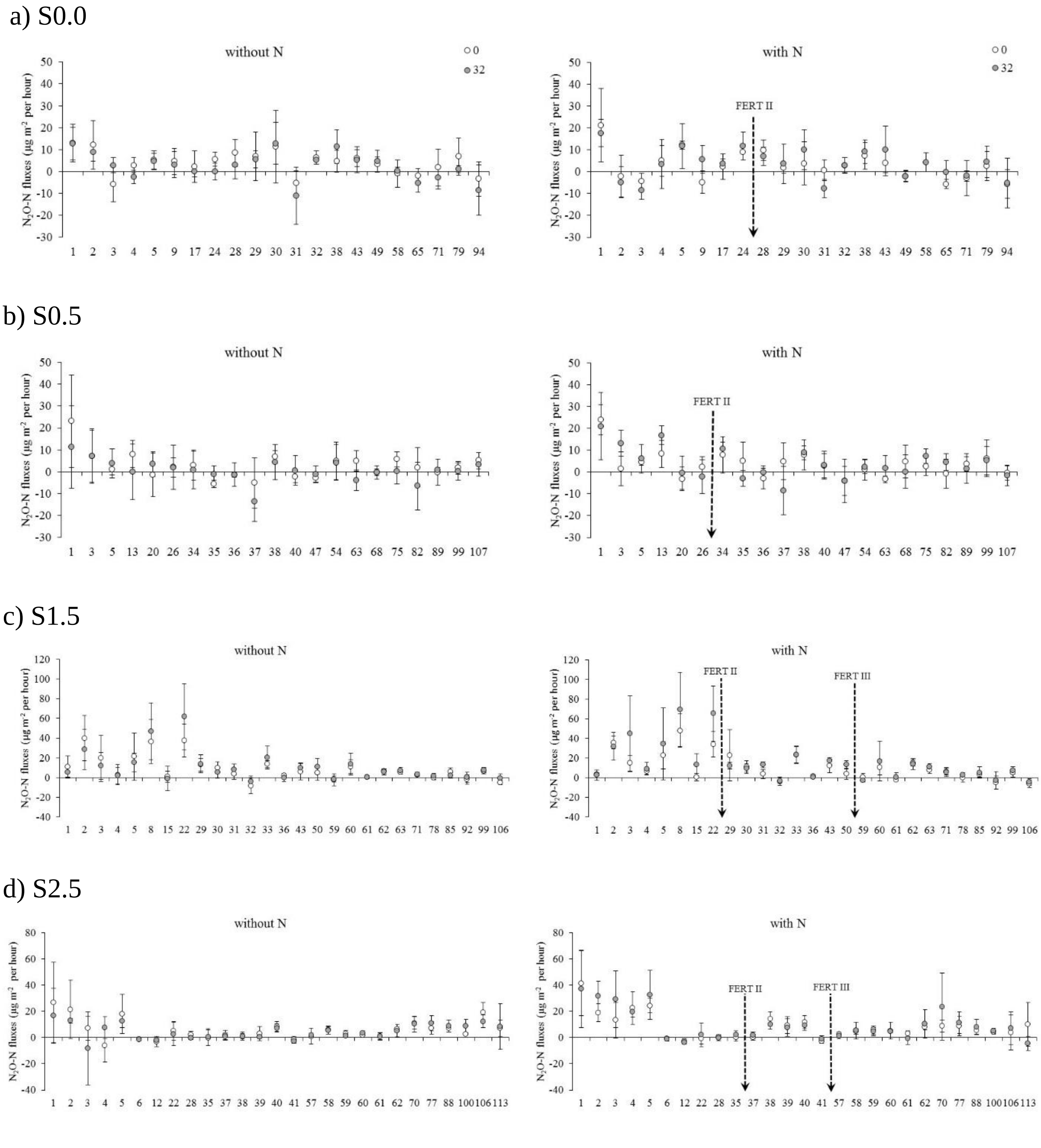

Figure 2. Estimated daily fluxes of N_2O-N from a clay Ferralsol without or with 90 kg N ha^{-1} (N) and without or with 32 Mg ha^{-1} biochar amendment, along four cropping seasons in the Brazilian Savannah. Seasons: irrigated common bean immediately after biochar application (a - S0.0); and aerobic rice at 0.5 (b - S0.5), 1.5 (c - S1.5) and 2.5 (d - S2.5) years after biochar application. Dots represent means and error bars represent corresponding standard errors (n = 4). Dotted arrows indicate the moment of N fertilizations that followed the first N fertilization at sowing event (FERT II, III: top dressings).

without biochar (41.43 ± 24.88), followed by the treatment with N and biochar (37.06 ± 29.43), without N and biochar (26.60 ± 30.95) and the treatment without N with biochar (16.80 ± 20.55).

3.2 Effects on averaged fluxes of N_2O-N and soil related variables within periods after N fertilization and along seasons

In none of the monitored seasons a significant effect of biochar on N_2O-N fluxes was observed (Table 2). Few effects of biochar were observed on soil related variables mainly along the latest seasons: S1.5 and S2.5. Significant effects on N_2O-N fluxes and soil related variables were mainly caused by N fertilization. In S0.0, only soil N-NO_3^- availability was significantly higher in the treatments with N fertilization, at least along FERT II and averaged over the entire SEASON (Fig. 4 - a).

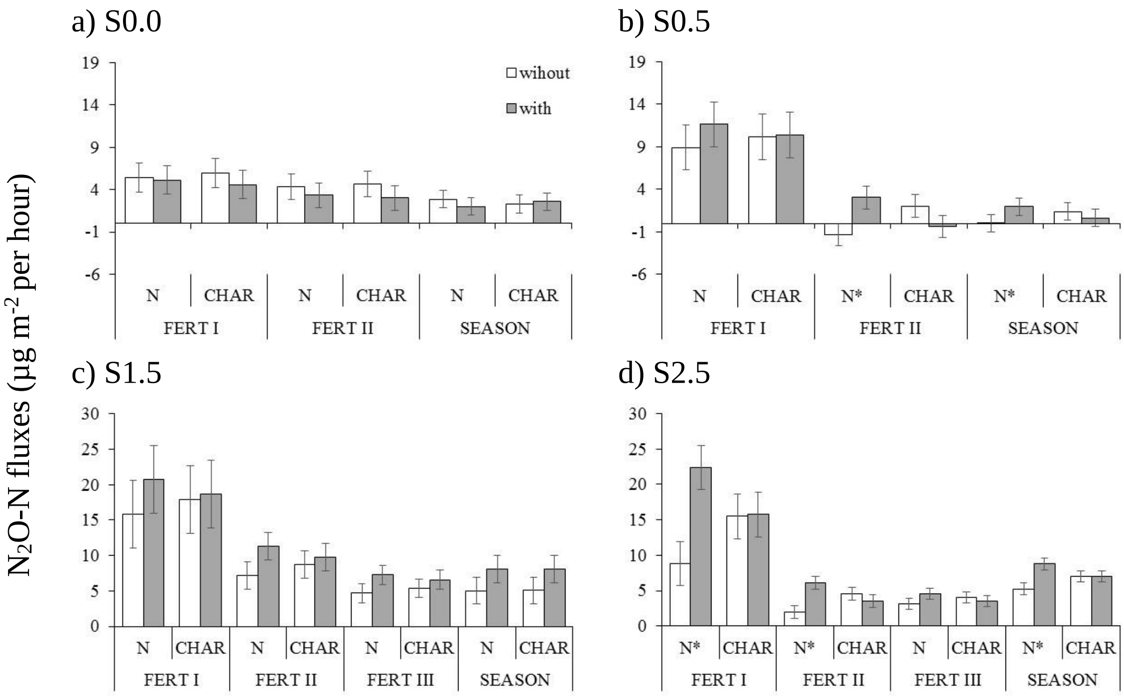

Figure 3. N_2O-N fluxes from a clay Ferralsol without or with 90 kg N ha^{-1} (N) and without or with 32 Mg ha^{-1} biochar amendment (CHAR) along 3 to 6 consecutive days after N fertilizations (FERT I: at sowing event; FERT II, III: top dressings) and the entire season (SEASON). Seasons: immediately (S0.0 - a), and at 0.5 (S0.5 - b), 1.5 (S1.5 - c) and 2.5 (S2.5 - d) years after biochar application. Columns represent estimated means fluxes and error bars the respective standard errors (n = 4). *Significant effects at 0.05 level (F tests for main effects N and CHAR are shown in Table 2).

Table 2. Nominal significance level (p values) arising from F tests for the effects of mineral N fertilization (N) and biochar (CHAR), and their interaction (N*CHAR), on N_2O-N fluxes and soil related variables along periods after N fertilization within four cropping seasons on a clay Ferralsol.

Effects	Effects by periods after N fertilization and season															
	FERT I				FERT II				FERT III				SEASON			
	N_2O-N	N-NH^+_4	N-NO^-_3	WFPS	N_2O-N	N-NH^+_4	N-NO^-_3	WFPS	N_2O-N	N-NH^+_4	N-NO^-_3	WFPS	N_2O-N	N-NH^+_4	N-NO^-_3	WFPS
S0.0																
N	0.9087	0.2479	0.5963	0.1727	0.6394	0.4300	**0.0046**	0.2565	nd	nd	nd	nd	0.4605	0.2075	**0.0081**	0.9362
CHAR	0.5813	0.3273	0.3088	0.4835	0.4383	0.2726	0.5798	0.9048	nd	nd	nd	nd	0.7876	0.8772	0.4548	0.5487
N*CHAR	0.6425	0.9343	0.8552	0.6426	0.7083	0.5769	0.4076	0.2565	nd	nd	nd	nd	0.1159	0.6985	0.5054	0.3153
S0.5																
N	0.4773	**0.0007**	**0.0170**	0.5552	**0.0218**	**0.0044**	**<.0001**	0.7477	nd	nd	nd	nd	**0.0408**	**<.0001**	**0.0001**	0.2685
CHAR	0.9592	0.8899	0.8990	0.8186	0.2008	0.6315	0.6908	0.6043	nd	nd	nd	nd	0.4012	0.7191	0.8314	0.4633
N*CHAR	0.3916	0.7724	0.6937	0.9235	0.7540	0.8237	0.6167	0.7477	nd	nd	nd	nd	0.3256	0.8515	0.5461	0.9359
S1.5																
N	0.4767	**<.0001**	**<.0001**	**0.0030**	0.1329	**<.0001**	**<.0001**	**0.0004**	0.1741	**0.0015**	**<.0001**	**<.0001**	0.0791	**<.0001**	**<.0001**	**<.0001**
CHAR	0.9015	0.8901	0.8644	0.2843	0.7048	0.4220	0.2983	**0.0301**	0.5419	0.4708	0.1138	**0.0004**	0.0804	0.1898	0.6637	**<.0001**
N*CHAR	0.3073	0.9461	0.5452	0.9862	0.5959	0.5081	0.7271	0.3881	0.8376	0.4844	0.1748	0.1131	0.5707	0.5212	0.6818	0.5093
S2.5																
N	**0.0031**	**<.0001**	**<.0001**	**0.0007**	**0.0024**	**0.0020**	**<.0001**	**<.0001**	0.2201	**<.0001**	**<.0001**	**0.0006**	**0.0024**	**<.0001**	**<.0001**	**<.0001**
CHAR	0.9555	0.2117	0.0829	**0.0004**	0.4029	0.4343	0.3147	**<.0001**	0.6484	0.8876	0.5078	**0.0003**	0.9767	0.1898	0.6637	**<.0001**
N*CHAR	0.3044	0.4318	0.2056	0.4364	0.9297	0.9153	0.1218	0.8887	0.7271	0.9285	0.7994	0.9018	0.3098	0.5212	0.6818	0.5093

N_2O-N: nitrous oxide fluxes (μg m^{-2} per hour); N-NO_3^-: available soil nitrate (mg kg^{-1}); N-NH_4^+: available soil ammonium (mg kg^{-1}); and WFPS: soil water filled pore space (%). Seasons: immediately (S0.0) and at 0.5 (S0.5), 1.5 (S1.5) and 2.5 (S2.5) years after biochar application.

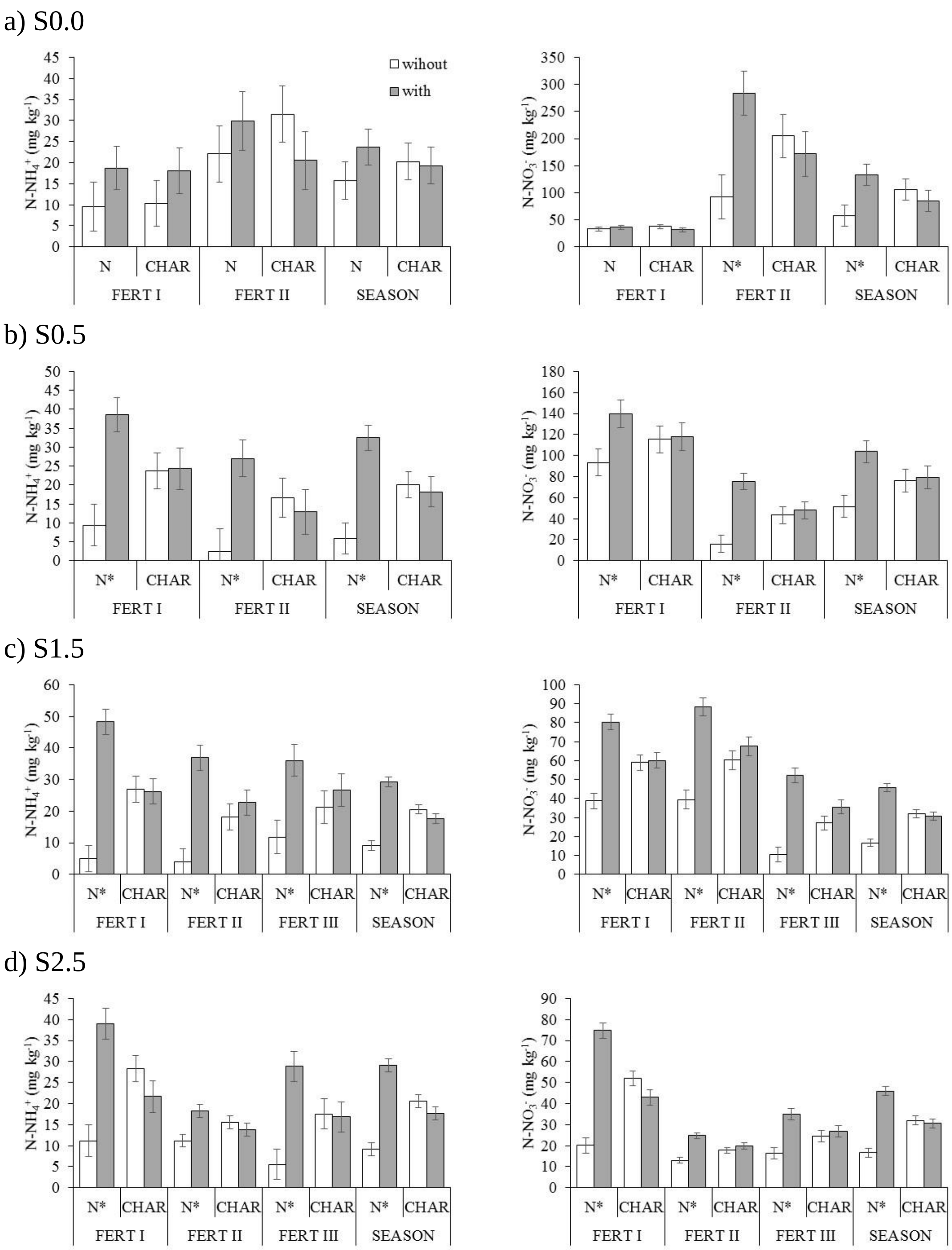

Figure 4. Soil ammonium ($N\text{-}NH_4^+$) and nitrate ($N\text{-}NO_3^-$) availability in a clay Ferralsol treated without or with 90 kg N ha^{-1} (N) and without or with 32 Mg ha^{-1} biochar amendment (CHAR). Data collected within 3 to 6 consecutive days after N fertilizations (FERT I: at sowing event; FERT II, FERT III: top dressings) and the entire cropping season (SEASON). Seasons: immediately (S0.0 - a), and at 0.5 (S0.5 - b), 1.5 (S1.5 - c) and 2.5 (S2.5 - d) years after biochar application. Columns represent estimated means and error bars the respective standard errors (n = 4). *Significant effects at 0.05 level (F tests for main effects N and CHAR are shown in Table 2).

Table 3. Estimated Pearson correlation coefficients for the relation between N_2O-N fluxes (µg m^{-2} per hour) and soil related variables in a clay Ferralsol without or with 90 kg N ha^{-1} (N) and without or with 32 Mg ha^{-1} biochar amendment (CHAR) and along four cropping seasons.

Treatments		Variables					
		N-NH_4^+	N-NO_3^-	WFPS	N-NH_4^+	N-NO_3^-	WFPS
		------------------S0.0-----------------			------------------S0.5------------------		
N	0	-0.12 (*0.5335*)	-0.13 (*0.4488*)	0.16 (*0.3451*)	0.05 (*0.8048*)	**0.33 (*0.0218*)**	-0.18 (*0.2241*)
	90	0.23 (*0.1804*)	0.04 (*0.8289*)	0.27 (*0.0995*)	0.02 (*0.8790*)	0.18 (*0.2358*)	-0.21 (*0.1544*)
CHAR	0	-0.06 (*0.7400*)	-0.15 (*0.3724*)	0.21 (*0.2064*)	0.06 (*0.6919*)	0.09 (*0.5504*)	-0.18 (*0.2240*)
	32	0.28 (*0.1158*)	0.02 (*0.9065*)	0.30 (*0.0653*)	0.18 (*0.3253*)	**0.53 (*0.0001*)**	-0.24 (*0.1045*)
nº of obs.		$40 \geq n \geq 30$			$48 \geq n \geq 29$		
		------------------S1.5-----------------			-------------------S2.5-----------------		
N	0	**-0.23 (*0.0154*)**	0.10 (*0.3078*)	0.15 (*0.1052*)	0.04 (*0.6716*)	-0.01 (*0.8879*)	**0.24 (*0.0050*)**
	90	0.14 (*0.1259*)	**0.26 (*0.0040*)**	**0.23 (*0.0109*)**	0.09 (*0.3291*)	**0.26 (*0.0029*)**	0.12 (*0.1750*)
CHAR	0	0.13 (*0.1722*)	0.14 (*0.1493*)	0.18 (*0.0604*)	**0.20 (*0.0182*)**	**0.23 (*0.0080*)**	0.14 (*0.1100*)
	32	0.14 (*0.1471*)	**0.31 (*0.0005*)**	0.10 (*0.3037*)	0.15 (*0.0821*)	**0.35 (*<.0001*)**	0.01 (*0.9352*)
nº of obs.		$120 \geq n \geq 109$			$136 \geq n \geq 129$		

Available soil ammonium (N-NH_4^+, mg kg^{-1}) and nitrate (N-NO_3^-, mg kg^{-1}), and water filled pore space (WFPS, %). Seasons: immediately (S0.0) and at 0.5 (S0.5), 1.5 (S1.5) and 2.5 (S2.5) years after biochar application. Values between brackets are nominal significance levels (p values).

In S0.5, N_2O-N fluxes, soil N-NH_4^+ and N-NO_3^- availability were significantly higher in treatments with N fertilization along FERT II and averaged over the entire SEASON (Fig. 3 - b, Fig 4 - b). Along FERT I, soil N-NH_4^+ and N-NO_3^- availability were also significantly higher in treatments with N fertilization, although no significant effects on N_2O-N fluxes were observed. The WFPS was not significantly affected by biochar or N treatments in S0.5 (Table 2, Fig. 5 - b). Fluxes of N_2O-N in treatments without N or with biochar amendment were positively correlated with soil N-NO_3^- availability in S0.5 (Table 3).

In S1.5, although significant effects of mineral N fertilization on soil related variables were present along all periods after N fertilization and averaged over the entire season, significant effects on N_2O-N fluxes were not observed. Soil N-NH_4^+ and N-NO_3^- availability were significantly higher in the treatment with N fertilization in any period measured and along the season in S1.5 (Fig. 4 - c). The WFPS was lower in treatments with N fertilization along all periods after N fertilization and averaged over the entire season (Fig. 5 - c). Conversely, the WFPS was higher in treatments with biochar amendment in FERT II and

FERT III, but lower along the entire season. Fluxes of N_2O-N in treatments with N fertilization or biochar amendment were positively correlated with soil N-NO_3^- availability (Table 3). In treatments with N fertilization, fluxes of N_2O-N were also positively correlated with WFPS. Conversely, in treatments without N fertilization, N_2O-N fluxes were negatively correlated with soil N-NH_4^+ availability in S1.5.

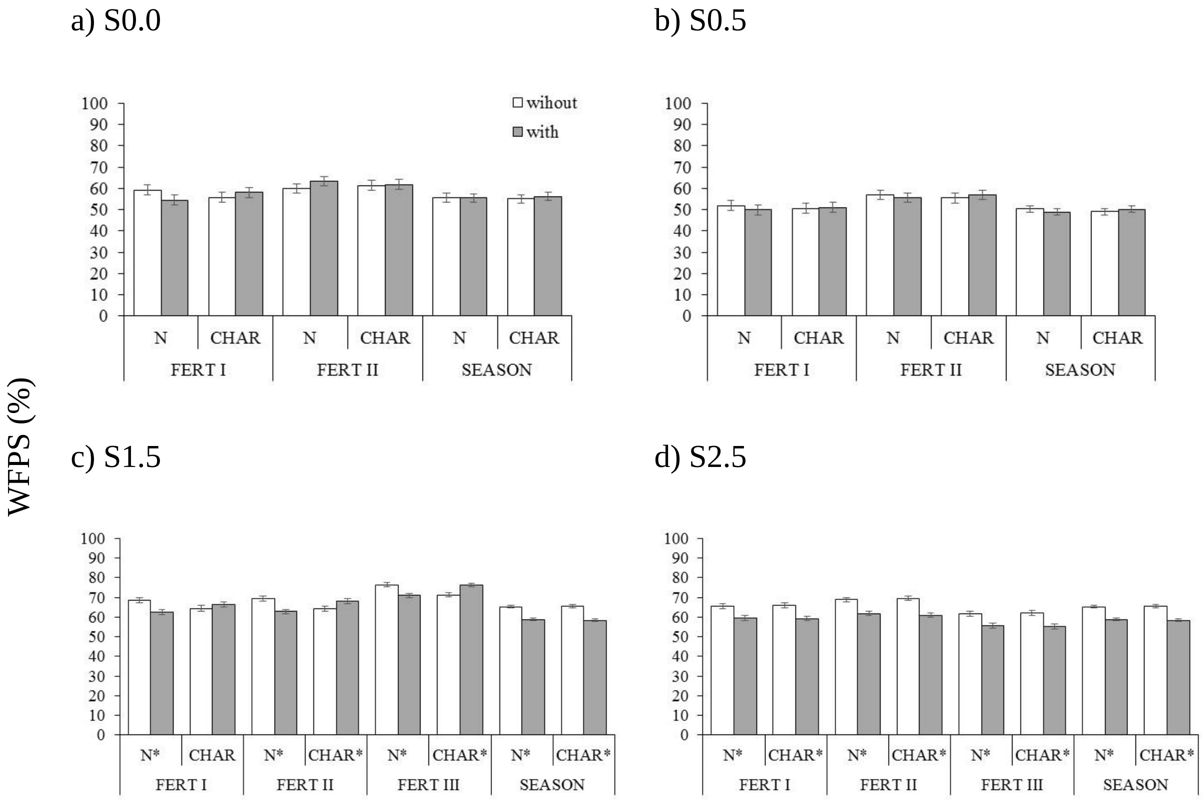

Figure 5. Soil water filled pore space (WFPS) in a clay Ferralsol treated without or with 90 kg N ha^{-1} (N) and without or with 32 Mg ha^{-1} biochar amendment (CHAR) along 3 to 6 consecutive days after N fertilizations (FERT I: at sowing event; FERT II, III: top dressings) and the entire season (SEASON). Seasons: immediately (S0.0 - a), and at 0.5 (S0.5 - b), 1.5 (S1.5 - c) and 2.5 (S2.5 - d) years after biochar application. Columns represent estimated WFPS means and error bars the corresponding standard errors (n = 4). *Significant effects (F tests for main effects N and CHAR are shown in Table 2).

In S2.5, significant effects of mineral N fertilization on N_2O-N fluxes and soil related variables were present along all periods after N fertilization and averaged over the entire season, except along the third period after N fertilization (FERT III), when N_2O-N fluxes were not significantly affected by mineral N fertilization (Table 2). The WFPS was significantly affected by biochar amendment along any period after N fertilizations. The WFPS was lower in treatments with biochar amendment or N fertilization along all periods after N fertilization and averaged over the entire cropping season (Fig. 5 - c). Conversely, soil N-NH_4^+ and N-NO_3^- availability were higher in treatments with N fertilization (Fig. 4 - c). Similarly, fluxes of N_2O-N were higher in treatments with N fertilization, except in FERT III (Fig. 3 - c). A positive correlation between fluxes of N_2O-N and soil N-NO_3^- availability was observed in treatments with N fertilization, irrespective of biochar amendment (Table 3). In treatments without biochar amendment, N_2O-N fluxes were also positively correlated with soil N-NH_4^+ availability; and in treatments without N fertilization, N_2O-N fluxes were positively correlated with WFPS.

3.3 Effects on soil pH, SOM and grain yield within cropping seasons

Soil pH was significantly affected by the mineral N fertilization within all four seasons monitored (Table 4). Usually, soil pH was lower in treatments with N fertilization (Table 5). The effect of the biochar amendment on soil pH was present and significant only in S0.0. In S0.0, soil pH was higher in the treatments with biochar amendment and the effect of biochar was significant within the treatment with N fertilization.

Table 4. Nominal significance level (p values) arising from F tests for the main effects mineral N fertilization (N) and biochar (CHAR), and their interaction (N*CHAR), on soil pH, soil organic matter (SOM) and grain yield (GY) along four cropping seasons on a clay Ferralsol.

Effects	Variables		
	pH (H_2O)	SOM (g kg^{-1})	GY (Mg ha^{-1})
	S0.0		
N	**<.0001**	0.1126	**0.0006**
CHAR	**0.0033**	0.0840	0.7580
N*CHAR	0.4399	**0.0313**	0.5910
	S0.5		
N	**0.0006**	**0.0587**	0.1219
CHAR	0.1660	0.7887	0.5485
N*CHAR	0.4362	0.9600	**0.0134**
	S1.5		
N	**<.0001**	**0.0287**	**0.0408**
CHAR	0.1776	0.3345	0.8254
N*CHAR	0.9521	0.8221	0.6361
	S2.5		
N	**0.0008**	0.2572	**<.0001**
CHAR	0.3102	**0.0170**	0.4108
N*CHAR	0.5914	0.6030	**0.0317**

Seasons: immediately (S0.0) and at 0.5 (S0.5), 1.5 (S1.5) and 2.5 (S2.5) years after biochar application. Mean values are shown in Table 5.

The SOM was significantly affected by the interaction of N and biochar in S0.0. In S0.5 and S1.5, the SOM was only affected significantly by the N fertilization and in S2.5, the SOM was significantly affected by biochar amendment (Table 4). In S0.0, the SOM was higher in the treatment with biochar amendment, if N fertilization was applied. In S0.5 and S1.5, the SOM was higher in the treatments with N fertilization and in S2.5, the SOM was higher in the treatments with biochar amendment (Table 5).

Table 5. Soil pH (H_2O), soil organic matter (SOM, g kg^{-1}) and grain yield (GY, Mg ha^{-1}) on a clay Ferralsol treated without or with 90 kg N ha^{-1} (N) and without or with 32 Mg ha^{-1} biochar amendment (CHAR) along four cropping seasons.

Treatments		Variables					
		Average			(p values)*		
N	CHAR	pH	SOM	GY	pH	SOM	GY
		S0.0					
0	0	4.63 (0.17)**	23.30 (0.88)	1.38 (0.36)	0.0630	0.6718	0.8652
	32	4.97 (0.14)	22.95 (0.74)	1.45 (0.33)			
90	0	3.49 (0.14)	20.95 (0.74)	2.95 (0.34)	**0.0067**	**0.0090**	0.5503
	32	3.99 (0.16)	23.41 (0.80)	2.70 (0.37)			
		S0.5					
0	0	4.78 (0.19)	15.57 (0.63)	3.96 (0.49)	0.6541	0.8237	**0.0268**
	32	4.85 (0.17)	15.44 (0.57)	2.89 (0.44)			
90	0	4.11 (0.18)	16.39 (0.58)	3.55 (0.45)	0.1363	0.8803	0.1157
	32	4.38 (0.20)	16.30 (0.66)	4.27 (0.52)			
		S1.5					
0	0	4.78 (0.14)	21.85 (1.13)	0.96 (0.28)	0.3104	0.4040	0.8576
	32	4.93 (0.14)	22.76 (1.01)	1.01 (0.24)			
90	0	3.98 (0.14)	23.88 (1.04)	1.48 (0.25)	0.3522	0.6021	0.6068
	32	4.11 (0.15)	24.45 (1.19)	1.35 (0.28)			
		S2.5					
0	0	4.56 (0.17)	24.62 (1.98)	1.33 (0.27)	0.7293	**0.0372**	**0.0386**
	32	4.50 (0.16)	29.74 (1.92)	0.67 (0.26)			
90	0	4.10 (0.16)	27.24 (1.92)	2.13 (0.26)	0.2798	0.1332	0.2721
	32	3.93 (0.17)	30.74 (2.05)	2.45 (0.28)			

Seasons: immediately (S0.0) and at 0.5 (S0.5), 1.5 (S1.5) and 2.5 (S2.5) years after biochar application.* Nominal significance levels (p values) arising from sliced F tests for the effects of CHAR within N.** Estimated means followed by corresponding standard errors (n = 4). Nominal significance values arising from F tests for the effects of N, CHAR and their interaction is shown in Table 4.

The grain yield (GY) was significantly affected by N fertilization in S0.0, S1.5 and S2.5. In S0.5 and in S2.5 the GY was significantly affected by the interaction of N and biochar (Table 4). In S0.0, S1.5 and S2.5 the GY was higher in treatments with N fertilization.

In S0.5 and S2.5 the GY within the treatments with N fertilization was lower in the treatment with biochar amendment (Table 5).

4. Discussion

Application of 32 Mg ha^{-1} biochar did not reduce N_2O-N fluxes (Fig. 3). Actually, there were no effects of biochar amendment on N_2O-N fluxes and soil related variables along all seasons monitored, except for WFPS along the last two seasons: S1.5 and S2.5 (Table 2). Most prominent effect of biochar along the seasons was an interaction with N-fertilizer on soil variables which express gradual changes in soil properties, such as soil pH and SOM, and consequently on GY (Table 5). Major effects on N_2O-N fluxes and soil related variables observed in this study were due to N fertilization. Usually, N fertilization enhanced N_2O-N fluxes, except in the two wettest seasons S0.0 and S1.5, when no effects of N fertilizer on N_2O-N fluxes were observed.

The season S0.0 was conducted under a continuously irrigated system and the season S1.5 was the wettest among the rain fed seasons (Fig. 1). Under this condition, most of the N lost to the atmosphere due to mineral N fertilization is likely to occur via N_2, resulting in a reduced probability to detect effects of mineral N fertilization on N_2O-N fluxes. In well-structured Ferralsols, aggregation favours aeration but can also create permanent anaerobic intra aggregate hot spots, where reduction of N_2O into N_2 occurs, as demonstrated by Leffelaar (1986). Immediately after biochar amendment, in S0.0, the soil pH increased (Table 5). A higher soil pH can favour activity of denitrifiers, which in turns increases the reduction of N_2O into N_2 as reported by Mukherjee et al. (2014) and Taghizadeh-Toosi et al. (2011). The WFPS was around 60% and similar for all treatments in S0.0 (Fig. 5 - a) and around 70-80% in S1.5 (Fig. 5 - c). Yet, the WFPS varied for both biochar and the N fertilizer treatments in S1.5: the use of N fertilizer reduced WFPS and the biochar amendment increased WFPS along periods after N fertilization. However, averaged over the entire season, both the N fertilizer and the biochar amendment reduced the WFPS. A reduced WFPS with N fertilization was probably a consequence of increased crop water uptake due to a greater plant biomass production with N fertilization, expressed as GY (Table 5). In both cropping seasons, S0.0 and S1.5, N fertilization increased soil N-NH_4^+ and N-NO_3^- availability and consequently crop yields. In both of these seasons, but most strongly in S1.5, positive correlations of N_2O-N fluxes with WFPS in treatments with N fertilizer, indicates that WFPS was a relevant soil variable for N_2O production and that denitrification was probably the dominant process for N transformations.

With time, changes in soil properties can lead to varying effects in N_2O-N fluxes from soil. A relative overall reduction in SOM level (Table 5), parallel to an overall decrease in WFPS (Fig. 5 - b), in S0.5 is one clear event along the seasons. Firstly, in S0.0, soil mechanization destroyed structure of the clay soil exposing native SOM to mineralization. Additionally, incorporation of 32 Mg ha^{-1} biochar to the soil, equivalent to about 1.45 Mg ha^{-1} of labile C (Table 1) that serves as prompt food for microbial activity, requires an N source for the microbial community to grow and reproduce. This is evident from the interaction effect of the N fertilizer and biochar on SOM in S0.0 (Table 4): if the N fertilizer was applied, then biochar amendment increased the SOM (Table 5). As soon as 2.5 years had passed, the SOM recovered to initial levels and the treatments with biochar amendment showed the highest SOM level in S2.5 (Table 5). Dynamics of SOM along seasons seems to play a relevant role on N_2O-N fluxes, which could be either due to mineralization of SOM or accumulation of SOM in the system. This is evident from the significant effect of N fertilizer on N_2O-N fluxes in S0.5 and S2.5 (Table 2). A parallel increment in soil organic C pool and N_2O emission with 12 Mg ha^{-1} corn-stalk straw biochar on a rice paddy field along 117 days was reported by Xie et al. (2013). Similarly, Liu et al. (2014) found an increase in SOM with 40 Mg ha^{-1} wheat straw biochar in a 5-year irrigated maize-wheat cropping seasons.

Over all seasons, the lowest magnitude of WFPS (around 50%) was observed in S0.5. Additionally, in S2.5, the WFPS was around 14% lower in treatments with N fertilizer, (Fig. 5 - d) and N_2O-N fluxes were positively correlated with soil N-NO_3^- availability in treatments with N fertilization (Table 3). Apart from the high soil mineral N availability, our results indicate that enhancement of N_2O-N fluxes in S0.5 and S2.5 might also result from variations in SOM levels and predominant aerobic-soil conditions. The interaction effect of N and biochar on GY in S0.5 and S2.5 indicates that there was N immobilization in the system. The GY in treatments without biochar was higher than with biochar amendment if N fertilizer was not applied (Table 5).

Contrariwise, Zhang et al. (2012a) found a reduction of around 51-56% in N_2O-N fluxes from a typical Chinese rice paddy soil. They observed this reduction at the first and second cropping seasons on a 39% clay soil after application of 40 Mg ha^{-1} wheat straw biochar together with 300 kg N ha^{-1}, compared to application of N fertilization alone. A reduction in N_2O emission from the N fertilizer applied with wheat straw biochar in rice paddy systems was also observed by Liu et al. (2012b). Most probably, this effect is related to an increase in soil aeration, when the soil was not completely flooded, due to a decrease in soil bulk density with biochar amendment. Likewise, Mukherjee et al. (2014) found a significant decrease in N_2O emission with 0.5% w/w oak wood biochar amendment on an artificially degraded silty-loam soil along the first 4 month of the soybean cropping season. They associated the reduction to increased soil aeration, sorption of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ on biochar surfaces or presence of inhibitory organic compounds that supressed microbial activity.

In our study, soil $N\text{-}NO_3^-$ availability was the soil related variable positively correlated with N_2O-N fluxes in treatments with biochar amendment in all three rain fed cropping seasons, in S0.5, S1.5 and S2.5, and to a lesser extent in S0.0 (Table 3). The strongest Pearson correlation coefficient was observed in S0.5, when WFPS was relatively the lowest over all seasons under assessment. Under aerobic conditions, such as in this study, nitrification is likely the main process for N_2O production, as observed by Carvalho et al. (2006). The presence of biochar can favour this process resulting in increased $N\text{-}NO_3^-$ availability and therefore N_2O emissions, as demonstrated by Sánchez-García et al. (2014). However, on the clay Ferralsol under the conditions of our study, the use of biochar amendment had no significant effect on N_2O-N fluxes from the N fertilizer applied. Similarly, Verhoeven and Six (2014) found no reduction in N_2O emission from a sandy clay loam soil treated with 10 Mg ha^{-1} walnut shell and pine chip biochar in a cropping system scale trial under Mediterranean climate. To the contrary, pine chip biochar enhanced N_2O-N fluxes related to the treatment with no amendment in two growing seasons after biochar application.

Along the four cropping seasons, negative fluxes were quite frequent (22-38%) (Fig. 2). Usually N_2O-N fluxes were periodic and few peaks over the cropping season can represent most of the losses, such as along the period after first N fertilization, right after the sowing event (Fig. 2, Fig. 3). Low or negative fluxes have been observed in the same area where this study was conducted (Carvalho et al. 2013a, Metay et al. 2007). The N_2O emission seems to be intrinsically low on tropical soils if compared to temperate soils (Jungkunst 2010). Over the cropping seasons, the highest flux was observed in the treatment with N fertilization and biochar amendment (69.35 ± 37.80 µg m^{-2} per hour) at 8 DAS along the third cropping season, 1.5 years after biochar application. The lowest flux was observed in the treatment without N fertilizer and with biochar amendment (-13.72 ± 9.12 µg m^{-2} per hour) at 37 DAS along the second cropping season, 0.5 year after biochar application.

Our results show that, regardless of biochar amendment, N fertilization was the major factor associated with N_2O-N fluxes in the cropping system. The N fertilization favours crop production, but can also enhance N_2O-N fluxes. Enhancement of N_2O emission seems trivial if compared to the gained benefits with N fertilization, such as increased SOM and crop yields (Table 4). On the other hand, although biochar application had no effect on N_2O-N fluxes, its application decreases crop yields if N fertilization is not present. Likewise, Zhang et al. (2012 b) found a significant increase in maize grain yield only if biochar and N fertilization were both applied. Our findings differ from Liu et al. (2014) who found a decrease in N_2O emission when 300 kg N ha^{-1} fertilizer was combined with 40 Mg ha^{-1} wheat straw biochar in a 5-year irrigated maize-wheat cropping experiment on a Chinese calcareous soil. At field scale, soil properties, such as pH, SOM and WFPS, and irrigation regimes might be the main causes for divergent results.

5. Conclusions

Application of 32 Mg ha^{-1} biochar amendment did not mitigate N_2O-N fluxes from the N fertilizer applied. The N fertilization enhanced N_2O-N fluxes, soil N-NH_4^+ and N-NO_3^- availability, especially along seasons characterized by lower WFPS. Fluxes of N_2O-N were periodic, generally with peaks at the period after first N fertilization. Effects of N fertilization and biochar amendment on soil pH, soil organic matter and water filled pore space along the four cropping seasons had an effect on dynamics of N_2O-N fluxes under the conditions of this study. Our findings point out the relevance of longer-term field studies on the impact of biochar on N_2O-N fluxes, which is likely to depend on gradual changes of soil properties and irrigation regimes.

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Chapter 6

General discussion

Márcia Thaís de Melo Carvalho

“Não sou nada.

Nunca serei nada.

Não posso querer ser nada.

À parte isso, tenho em mim todos os sonhos do mundo.”

(Tabacaria, Álvaro de Campos, heterônimo de Fernando Pessoa, 1944)

The general research question addressed in this study was: *can biochar be used in an agronomically beneficial and sustainable manner to increase the productivity of aerobic rice production systems in a tropical Savannah?* We analyzed effects of a single application of wood biochar on soil chemical and physical properties and on aerobic rice yields along four cropping seasons on a clay and a sandy soil type. We also studied environmental implications of the use of biochar, particularly its influence on soil water retention capacity and N_2O emissions. Here we will discuss the main findings of this thesis and the methodological and statistical innovations used to realize this study. Finally, we will reflect on the potential of biochar to contribute to sustainable intensification of future farming systems and on the scientific challenges that still need to be addressed in forthcoming research.

1. Main findings

Table 1 summarises the main effects of biochar on key soil properties, crop yield and N_2O emissions along growing seasons after biochar application as reported in the previous chapters. We will start this section with the effects of biochar on soil physical and chemical properties. We will then discuss the impact of biochar on rice yields and consider the effect of this soil amendment on N_2O emission. The effect of biochar on soil water holding capacity varied according to soil type. On the sandy soil, biochar increased the water retention capacity in both seasons under assessment, 2 and 3 years after its application (*Chapter 3*). Conversely, biochar amendment decreased the water retention capacity of the clay soil at 1.5 and 2.5 years after its application, with most significant effects on soil water potential higher than -60 kPa in both seasons (*Chapter 4*). Many studies have reported an increased water retention capacity of a sandy soil after biochar application, but most often these results were obtained in pot experiments (*Chapter 1*). There are however also some field studies reporting on positive effects of biochar on water retention capacity of sandy loam soils. Liu et al. (2012a) reported a twofold increment in water retention capacity of a sandy soil treated with biochar and compost. However, Hardie et al. (2014) and Liu et al. (2012a) argue that, at field scale level, significant differences are not easily detectable due to the high spatial variability intrinsic to field experiments. In our study, we also proposed a more sensitive analysis to this type of experiment using nonlinear mixed models, which will be discussed in the next section of this chapter. Even though biochar application increased the water retention capacity of the sandy loam soil at 2 and 3 years after its application, a positive crop response was not observed in these two growing seasons. It was made plausible, that the absence of a crop response was due to the extraordinarily wet conditions during the last two seasons under assessment (*Chapter 3*). With 32 Mg ha^{-1} (or 1.5% w/w) of biochar amendment, the increment in plant available water was 26%. This represents a relevant increment of ~ 14 mm in the upper (5-10 cm) soil layer. This additional amount of water would be sufficient to satisfy the crop demand for approximately three additional days without rainfall. Such an increment is relevant in an environment where in principle there is enough rain, but in which dry spells of five to seven days frequently cause severe yield losses.

Table 1. Overview on effects of biochar amendment on key soil properties and grain yield along cropping seasons on a sandy and clay soil in the Brazilian Savannah.

	-------------------Sandy------------------		-------------------Clay--------------------	
	First 2 seasons	Latter seasons	First 2 seasons	Latter seasons
Soil water retention capacity	?	↑	?	↓
Soil organic matter	↑	x	x	↑
Soil acidity	↓	↓	↓	↓
Grain yield	↑	x	x	↓
N_2O emission	?	?	x	x

↑ = increased; ↓ = decreased; x = no effect; ? = not investigated.

The effect of biochar on decreasing water retention capacity of the clay soil is probably a consequence of the intrinsic low bulk density of the material and the low capacity of charcoal to retain water if compared to clay particles (*Chapter 4*). Our findings are in agreement with Tryon (1948) and, to our best knowledge; this is the first time that a field study confirms such an effect. So far, most of the reports on effects of biochar on water retention capacity of a clay soil mention the absence of an effect or a positive response. The response seems to be strongly dependent on the amount of biochar added and the conditions in which biochar is applied to the soil, i.e., tillage or no-tillage, pot or field experiment (*Chapters 1*, *4*). Further, soil tillage for biochar incorporation and crop management along the cropping seasons also seem to have an effect on the physical structure of a clay soil, affecting its water retention capacity. Although biochar decreased the water holding capacity of the clay soil, the soil organic matter (SOM) increased with biochar rate in 2.5 and 3.5 years after its application. According to Zimmerman et al. (2011), SOM sorption to biochar can occur, with time, either onto external biochar surfaces or within biochar pores. The biochar used in our study has properties, such as the presence of functional groups and porosity that can favour both mechanisms. Liu et al. (2014) also found an increment in soil organic C with biochar application along five cropping seasons after its application. They attributed this result to a high microbial stability of biochar derived C in soil, without causing a priming effect for indigenous soil organic C. In our study, the effect of biochar on SOM seems, at least partly, to be also dependent on system management. The fluctuation of SOM levels along seasons in control treatments indicates that with additional crops in between rice seasons, as was the case on the clay soil, the effect of biochar on SOM was positive with time (Table 2). It also shows that around 0.5 year after biochar application in the clay soil and 1 year after biochar application in the sandy soil, the SOM level dropped.

Table 2. Response models representing the quantitative effects of biochar (char) and mineral N fertilization (N) on soil organic matter within 0-20 cm soil layer along cropping seasons after single biochar application in a sandy and clay soil of the Brazilian Savannah.

Season	Fitted models	R^2
	Sandy soil	
S0†	$12.45 + 0.00825\ char^{*}$	0.21
S1†	$7.46 - 0.0528\ char^{*} + 0.0019\ char^{2\ **} + 0.0393\ N^{***} - 0.0003N^{2\ ***}$	0.79
S2	$9.92 - 0.0684\ N^{**} + 0.00068\ N^{2\ **}$	0.50
S3	$9.65 + 0.1925\ N^{**} - 0.00204\ N^{2\ **}$	0.38
	Clay soil	
S0.0	19.95	0.00
S0.5	$15.56 + 0.00894\ N^{*}$	0.15
S1.5	$25.39 - 0.1338\ char^{ns} + 0.005224\ char^{2\ *}$	0.12
S2.5	$25.50 + 0.06684\ char^{*}$	0.16
S3.5	$29.87 + 0.06892\ char^{**}$	0.37

Biochar rate: 0, 8, 16, 32 Mg ha^{-1}, N rate: 0, 30, 60, 90 kg ha^{-1}; R^2: squared Pearson correlation coefficient between observed and predicted values. Degree of evidence of parameter estimates: $^{***}p \leq 0.01$, $^{**}0.01 < p \leq 0.05$, $^{*}0.05 < p \leq 0.10$, $^{ns}\ p > 0.10$. †Adapted from Petter et al. (2012).

In the sandy soil, where after biochar application there was fallow in between rice seasons, the effect of biochar on SOM was only significantly positive in the first two cropping seasons. This short-term effect of biochar on SOM in the sandy soil is most likely a result of direct addition to the soil of labile C (around 3% of the total C) present in the wood biochar. The mineralization of organic residues that were incorporated to the soil for establishment of the field trial might also have a part on increasing the SOM level. However, with time, the additional SOM is unlikely to be a result of the mineralization of crop residues added along the seasons. In the clay soil, for example, a simple calculation about order of magnitude of root biomass production and possible increase therein from biochar, indicated that only around 7% per season of the total increase in SOM can be attributed to increased root mass production. Although we could not clarify in our study mechanisms behind increased SOM with biochar application, this is an exciting finding because it points out that biochar amendment can serve as a promising soil management capable to sequester useful C in soil. The useful C in this case is not only the pyrogenic C that is resistant to mineralization in time, but also the C that accumulates in form of organic matter, a source of energy for soil life. Although there is much uncertainty about the relation between SOM and crop productivity (Lal 2009), most evidence suggests that SOM has a positive effect, especially in soils with low C content (Lal 2006) such as in this study. The increment in SOM can have indirect positive effects on crop yields by improving soil quality, such as improved plant nutrition via increased soil capacity to exchange cations (*Chapter 4*). It is very clear that a positive effect of biochar on increasing the soil pH (liming effect) and K availability was present in both soil types along seasons (Table 3).

In the clay soil, the "liming" effect was significantly present up to 1.5 years after biochar application and in the sandy soil even up to 3 years after biochar application. The positive effect on K availability was present up to 3.5 years after biochar application in the clay soil and up to 3 years after biochar application in the sandy soil. As a conventional liming operation, the effect of biochar application seems to be temporary, especially in the acidic clay Ferralsol, requiring frequent application. This effect of biochar is especially important for the soils of the tropical Savannahs, which requires correction of pH to become agronomically productive. Correction of the soil pH in the Brazilian Savannah was a revolutionary technique, enabling this region to produce over 40% of the total production of major crops in Brazil nowadays (*Chapter 1*). This thesis shows that biochar can act as a source of K, Ca and Mg increasing soil pH, from which many crops can benefit. A price comparison between biochar and conventional sources of Ca and Mg should show if biochar is a cheaper alternative. This aspect and other agronomic and scientific implications of this study will be discussed in the last section of this chapter. The crop response to biochar application varied according to the changes observed in soil chemical and physical properties and to the weather conditions along the seasons. Table 4 shows the effect of biochar on crop yields along seasons after its application in both soil types.

On the clay soil, biochar had no effect on common bean yields immediately after its application (S0.0). Similarly, biochar amendment had no effect on rice yields at 0.5 (S0.5) year after biochar application (*Chapter 2*). At 1.5 (S1.5) and 2.5 (S2.5) years after biochar

Table 3. Response models representing the quantitative effects of biochar (char) and mineral N fertilization (N) on soil pH and K availability within 0-20 cm soil layer along cropping

seasons after single biochar application in a sandy and clay soil in the Brazilian Savannah.

Variable	Season	Fitted models	R^2
		----Sandy soil----	
pH	S0†	$5.65 + 0.009193\ \text{char}^{***} + 0.002573\ \text{N}^{***}$	0.80
	S1†	$5.26 + 0.003828\ \text{char}^{***} + 0.002906\ \text{N}^{***}$	0.85
	S2	$4.72 + 0.005402\ \text{char}^{***} - 0.00396\ \text{N}^{**} + 0.000031\ \text{N}^{2\,*}$	0.64
	S3	$4.87 + 0.00262\ \text{char}^{**} - 0.00541\ \text{N}^{***} + 0.000062\ \text{N}^{2\,***}$	0.80
K	S0†	$105.76 + 0.8587\ \text{char}^{***} - 0.5532\ \text{N}^{**} + 0.006858\ \text{N}^{2\,**}$	0.68
	S1†	$103.27 - 0.8487\ \text{char}^{ns} + 0.04457\ \text{char}^{2\,*}$	0.49
	S2	$59.19 + 0.9749\ \text{char}^{**} + 0.2660\ \text{N}^{*} - 0.01406\ \text{char}\times\text{N}^{*}$	0.38
	S3	$44.10 + 0.3332\ \text{char}^{***}$	0.42
		----Clay soil----	
pH	S0.0	$4.81 + 0.01512\ \text{char}^{***} - 0.00998\ \text{N}^{***}$	0.78
	S0.5	$5.06 + 0.005716\ \text{char}^{**} - 0.00312\ \text{N}^{**}$	0.70
	S1.5	$5.16 + 0.00394\ \text{char}^{*} - 0.00643\ \text{N}^{***}$	0.86
	S2.5	$5.10 - 0.00518\ \text{N}^{***}$	0.64
	S3.5	$5.68 - 0.01127\ \text{N}^{***} - 0.01682\ \text{char}^{*} + 0.000532\ \text{char}^{2\,**}$	0.94
K	S0.0	$92.18 + 1.8709\ \text{char}^{***} - 0.3835\ \text{N}^{***}$	0.89
	S0.5	$59.37 + 1.3705\ \text{char}^{***} - 0.1011\ \text{N}^{ns} - 0.0127\ \text{char}\times\text{N}^{**}$	0.78
	S1.5	$70.42 + 0.6971\ \text{char}^{***} - 0.4460\ \text{N}^{***}$	0.86
	S2.5	$94.03 + 0.5852\ \text{char}^{***} - 0.6318\ \text{N}^{***}$	0.86
	S3.5	$68.55 + 1.4102\ \text{char}^{***} - 0.4161\ \text{N}^{***} - 0.01619\ \text{char}\times\text{N}^{**}$	0.89

Rate of biochar: 0, 8, 16, 32 Mg ha^{-1} and N: 0, 30, 60, 90 kg ha^{-1}; R^2: squared Pearson correlation coefficient between observed and predicted values. Degree of evidence of parameter estimates: $^{***}p \leq 0.01$, $^{**}0.01 < p \leq 0.05$, $^{*}0.05 < p \leq 0.10$, $^{ns}\ p > 0.10$. †Adapted from Petter et al. (2012).

application, the rice yield decreased with biochar rate and was dependent on the rate of N fertilization applied: rice yield only increased with biochar rate if more than 60 kg N ha^{-1} was applied. At 3.5 (S3.5) years after biochar application, no effect on rice yield was observed as discussed in *Chapter 4.* To interpret the effects of biochar on rice yield due to its effects on soil water retention capacity and soil chemical properties was challenging. Yield components were a good indicator of the effects of biochar on N and water availability along rice growing seasons. The negative effect of biochar on rice yield arises from both negative effects on water retention capacity and soil N availability in the clay soil. On the sandy soil, biochar had no effect on grain yield at 2 (S2) and 3 (S3) years after its application, contrary to the prominent positive effects observed immediately (S0) and one year (S1) after biochar application. Interestingly, these two first seasons reported by Petter et al. (2012) were very dry and the latest two seasons reported in this study were very wet (*Chapter 3).* Due to this unfortunate sequences of dry and wet seasons it remains impossible to directly test if the positive effects observed by Petter et al. (2012) were due to fertilization (nutrients added by the biochar) or due to improved water retention, or maybe both. This is why our analysis of the soil water retention curves was valuable, it provides the missing link, it presents strong indirect evidence that the positive biochar effect was (at least partially) due to increased water retention. Nevertheless, application of biochar in this sandy loam soils seems a strategic option to avoid yield losses and even maintain yield stability in case of dry spells or dry seasons. Throughout the latest 35 years (from 1979 to 2013) average precipitation rate during the months of January and February in the municipality where the field trial is located was 507 mm (Agritempo 2014). Further, the frequency of an amount of rainfall lower than 400 mm was 29%, considering that, a minimum water supply of 400 mm, well distributed along the entire growing season, is required in order to avoid yield losses in aerobic rice systems (Crusciol et al. 2013).

Table 4. Response models representing the quantitative effects of biochar (char) and mineral N fertilization (N) on grain yield along cropping seasons after single biochar application in a sandy and clay soil in the Brazilian Savannah.

Season	Fitted model	R^2
	Sandy soil	
S0†	$0.52 + 0.017\ \text{char}^{***}$	0.99
S1†	$0.50 + 0.017\ \text{char}^{***}$	0.99
S2	1.15	0.00
S3	$0.49 + 0.002156\ \text{N}^{*}$	0.20
	Clay soil	
S0.0‡	$1.49 + 0.02727\ \text{N}^{***} - 0.00014\ \text{N}^{2\ **}$	0.93
S0.5	$2.07 + 0.03718\ \text{N}^{**} - 0.00036\ \text{N}^{2\ **}$	0.40
S1.5	$0.78 - 0.00668\ \text{char}^{*} + 0.00512\ \text{N}^{**}$	0.56
S2.5	$0.73 - 0.01733\ \text{char}^{**} + 0.0099\ \text{N}^{***} + 0.00034\ \text{char}{\times}\text{N}^{***}$	0.86
S3.5	$2.13 + 0.0096\ \text{N}^{*}$	0.12

Rate of biochar: 0, 8, 16, 32 Mg ha^{-1} and N: 0, 30, 60, 90 kg ha^{-1}; R^2: squared Pearson correlation coefficient between observed and predicted values. Degree of evidence of parameter estimates: $^{***}p \leq 0.01$, $^{**}0.01 < p \leq 0.05$, $^{*}0.05 < p \leq 0.10$, ^{ns} $p > 0.10$. †Adapted from Petter et al. (2012); ‡ Grain yield of irrigated common bean.

In this study, the impact of biochar on N_2O emission was presented for the clay soil only. We found no effect of biochar amendment on N_2O-N fluxes from the clay soil (*Chapter 5*). The mineral N fertilization increased N_2O emissions. Gradual changes in SOM and water filled pore space (WFPS) seems to have a greater impact on magnitude of N_2O-N fluxes along seasons. The lack of a significant effect of biochar amendment on N_2O-N fluxes is probably linked to the physical structure of the clay Ferralsol and the aerobic nature of the cropping system. Under this well-drained condition, there is no accumulation of water on the soil surface. Further, the clay Ferralsol is more than 1 m deep, and leaching might be high. Major part of the mineral N applied as top dressing can be lost via volatilization (Carvalho et al. 2013). The remaining part, which is not taken up by the crop, is nitrified to nitrate and denitrified to N_2O or N_2 under anaerobic conditions. As wood biochar reduced WFPS, mainly along the latest seasons, anaerobic conditions were unlikely to occur, except immediately after biochar application, in S0.0. In S0.0, the system was managed with irrigated common beans, and irrigation was applied at every 3 days. Although evaporation is high along the winter in the Brazilian Savanah, the irrigation might have provoked anaerobic conditions and preferential losses of mineral N via N_2. Overall, fluxes were very low over the seasons. Six et al. (2014) also observed no effect of biochar on N_2O-N fluxes under aerobic conditions. According to Sanchez-Garcia et al. (2014), under aerobic conditions, where most of the N_2O-N fluxes are produced due to favourable nitrification, biochar application might not result in a reduction of N_2O-N fluxes. To the contrary, biochar application might even enhance N_2O-N fluxes. Conversely, under anaerobic conditions, such as in flooded rice systems, where N_2O-N fluxes are produced due to favourable denitrification, biochar might reduce N_2O emissions due to a decrease in soil bulk density. Liu et al. (2012b) and Zhang et al. (2012) observed this reduction in rice paddy field experiments. Our study points out the relevance of irrigation regimes and soil physical and chemical properties in order to determine effects of biochar on N_2O-N fluxes along seasons under field conditions.

2. The methodological and statistical approach

Considering the importance of field trials in agricultural sciences (Spiertz 2014), adequacy of statistical methods to deal with the intrinsic spatial variability, patterns of spatial correlations and with repeated measurements in space and time are crucial in order to deliver unbiased and more precise outcomes. Here we discuss the challenges related to statistical analysis of field trials derived from constraints in randomization due to operational features. Advantages and disadvantages of field trials versus laboratory (artificially controlled) studies is also explored. Finally, we present the novelty of the method applied to analyse data from the field trials used in this study.

This study was based on data from two field trials, each one located in one site within the Brazilian Central West region on a sandy and a clay soil. Both experiments were designed with same treatments but with differences in field layout (experimental design) due to operational constraints. The experiment on the clay soil was established under a centre pivot for sprinkler irrigation and the experiment on the sandy soil was only rain fed. In both experiments, it was not possible to use a randomized block design due to randomization constraints. In each, there were four replicates per treatment (N x char combinations). However, on the sandy soil, the N fertilization levels were systematically allocated into strips instead of completely randomized within blocks due to limitations in area and requirements of mechanization process for sowing and fertilizer application. The same was done on the clay soil; however, in this case, plots had to be placed according to the pivot layout avoiding places nearby the wheels. Instead of a design-based approach, we adopted a contemporary statistical model based approach to overcome constraints derived from the lack of randomization in a classical experimental design (Schabenberger and Pierce 2002). This was done via linear mixed modelling of the relationship between the measured soil and plant variables and biochar and N-fertilization rate. A mixed-effects model (mixed model) comprises both fixed and random effects thus permitting inclusion of random effects, which account for times correlations and patterns of spatial dependencies (Littell 2006). In the case of the sandy soil the random effects considered were within blocks and rows within blocks because the strips corresponded to N levels (Fig. 1). On the clay soil, random effects were location of plots in columns and rows (Fig. 2). The mixed model was a sound alternative for adequate analysis in both cases, although differences in experimental design did not allow a conjoint analysis of the effects of biochar and N fertilization for both soil types. The analysis on the effects of biochar and N fertilization on soil chemical and physical properties and on aerobic rice yields was performed separately for the clay soil in *Chapters 2* and *4* and for the sandy soil in *Chapter 3* of this thesis.

An innovative approach developed in this study was the use of nonlinear mixed modelling to fit and compare soil water retention curves. The nonlinear mixed model allows testing differences among biochar treatments considering the overall variance of soil moisture arising from within treatments variance. Such formal testing is not possible when soil water retention curves are fitted for each biochar treatment individually by commonly used specific software such as the one proposed by Dourado-Neto et al. (2000). In our soil water retention curve modelling, we also used plot as random effect aiming to account for correlations among repeated measurements taken within the same soil sample. The idea of applying such approach derive from a study by Omuto et al. (2006), who demonstrated the relevance of considering the sources of the intra/inter relationships between individual points using mixed effects modelling with environmental correlates. They showed that neglecting the high variability of soil hydraulic parameters between and among soil units during modelling of the functional soil processes could lead to potential errors. The use of nonlinear mixed model to estimate parameters of soil water retention curves is presented in *Chapter 3* of this thesis.

In our study about the impact of biochar on N_2O-N fluxes, plot was again included as a random effect in a mixed model, once repeated fluxes and related soil measurements were taken in the same plot along cropping seasons, as presented in *Chapter 5*. Further, the mixed model allows comparison of treatments and trends over time. According to Littell et al. (1998), this is especially important for analysis of repeated measures data because measurements taken close in time are potentially more highly correlated than those taken far apart in time. Usually this aspect is not taken into account in this type of field study, where N_2O-N fluxes are assessed along a period of time, with daily or weekly intervals. Fluxes are measured via static chambers that are placed in the same spot along the field trial. In our study, we considered that measurements taken in the same chamber along the entire cropping season were correlated because they belong to a plot that is unique in itself and analysis within periods after N fertilization were also applied. According to Jungkunst et al. (2012), N_2O-N fluxes are very sensitive to temporal and spatial variability and models show even higher sensitivity. To account for spatial variations of N_2O-N fluxes in a regional scale is yet a challenge.

We believe our approach using mixed models is a novel contribution for agronomy research. Whereas precision and control of random effects increases under artificially controlled conditions, the opposite occurs under field trial conditions, where usually there will always be random effects to account for, i.e., consideration of environmental covariates in the estimation process will be always present. Yet, in order to develop new agronomic technologies the assessment on a field trial scale is essential, because it can mimic real farming conditions. On the other hand, laboratory studies are indispensable to deliver timely results and insights prior to planning of a field trial. Research on biochar needs to advance from artificially controlled conditions to farming conditions in order to deliver results that are more relevant and representative to real conditions (Glaser et al. 2002, Sohi et al. 2010, Jeffery et al. 2011, Liu et al. 2013, Mukherjjee and Lal 2014). This was the challenge of this thesis: to evaluate a relatively long-term effect of the use of wood biochar as a soil amendment under typical cropping systems of the Brazilian tropical Savannah.

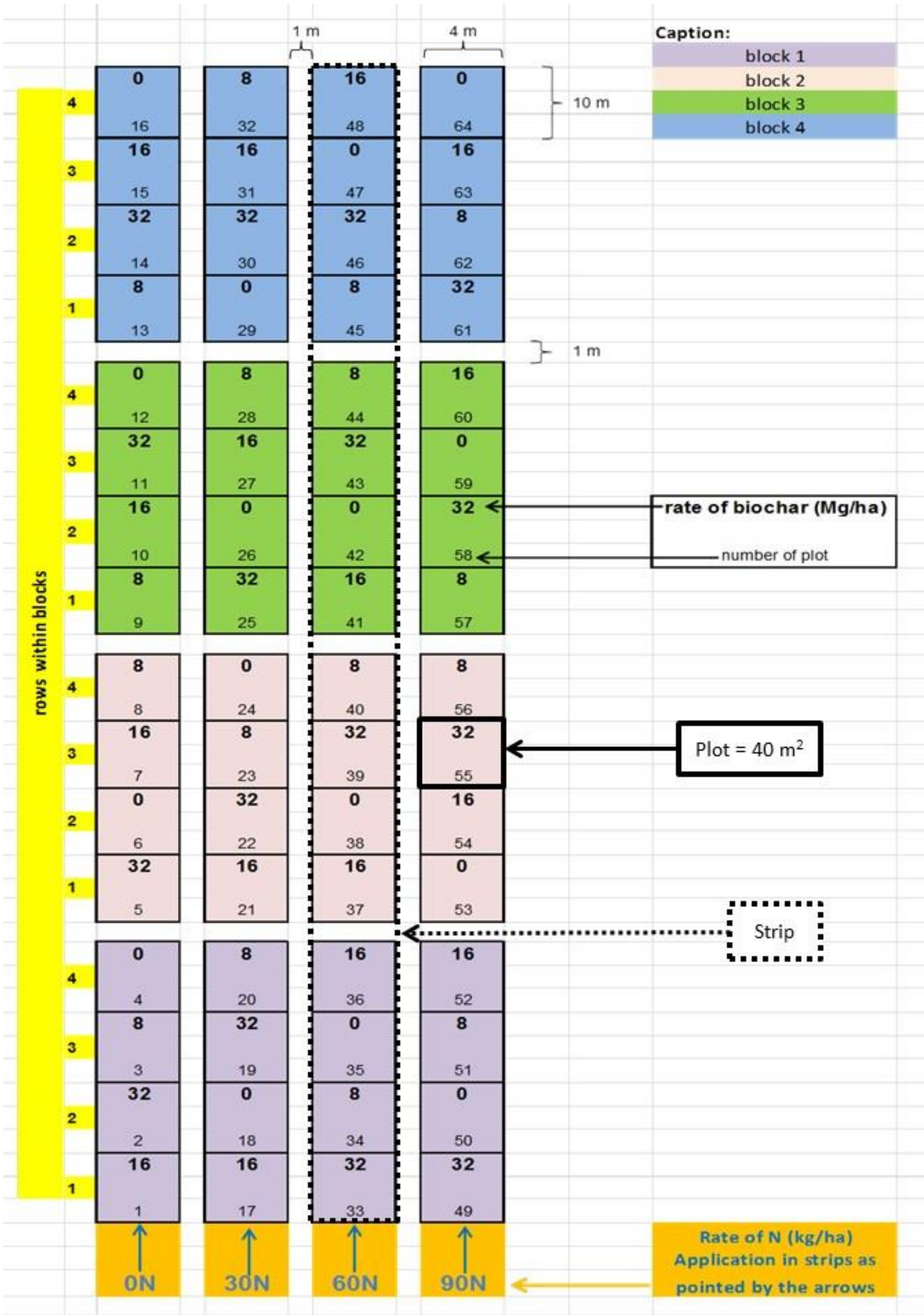

Figure 1. Experimental design on the sandy soil at Estrela do Sul Farm in Nova Xavantina, Mato Grosso, Brazil.

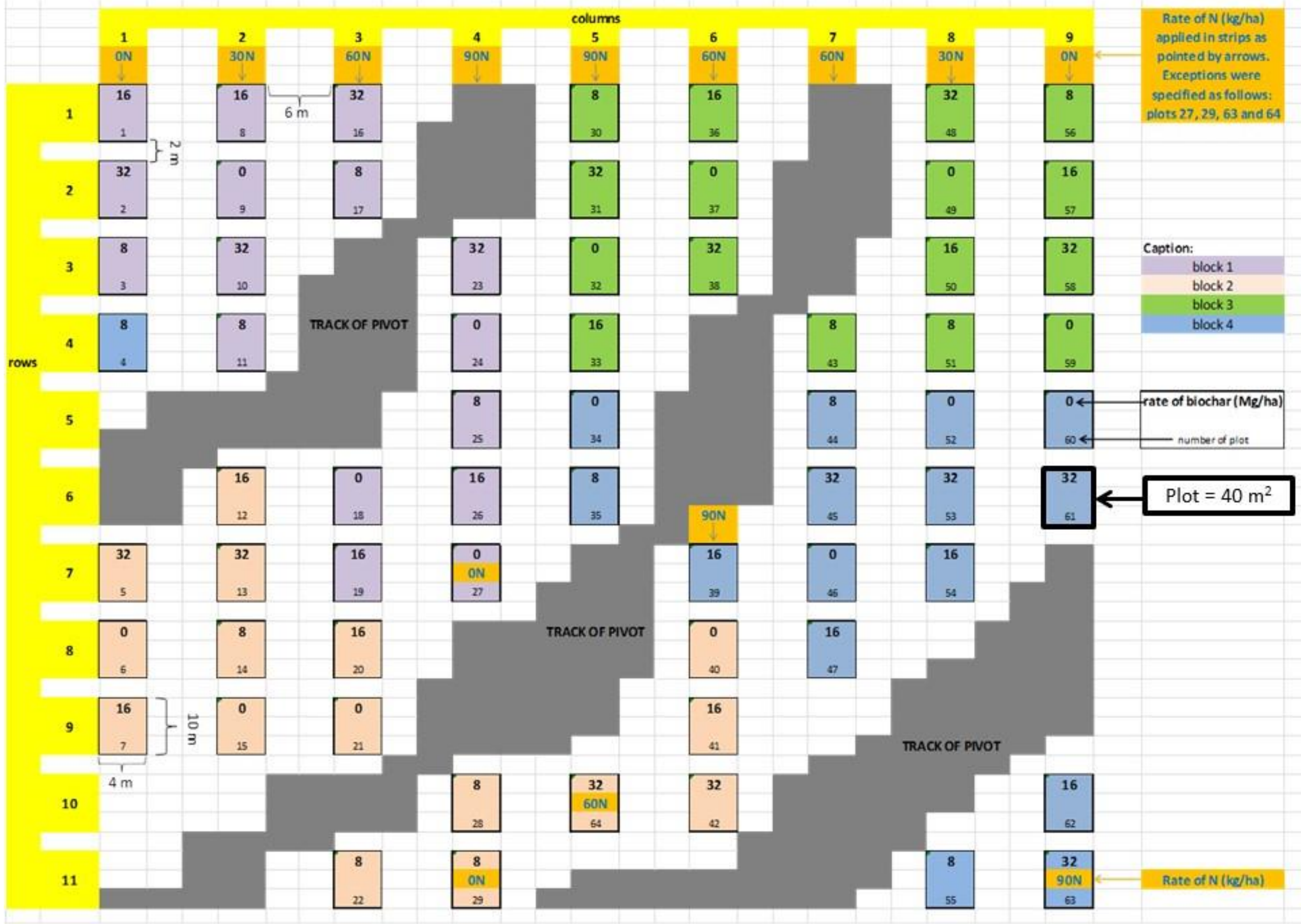

Figure 2. Experimental design on the clay soil at Capivara Farm in Santo Antonio de Goias, Goias, Brazil.

3. Contribution of biochar to sustainable intensification of farming systems in the Brazilian Savannah

Biochar amendment can be used as an alternative for increasing the water retention capacity of sandy loam soils. The improvement of water retention capacity seems to rely strongly on the characteristics of the biochar used, such as specific surface area, which in turn depends on the feedstock, temperature and time of pyrolysis. In order to obtain fruitful results, timely physic-chemical characterization of the potential biochar to be used is advised. Properties such as hydrophobicity, specific surface area, number and size of pores are the most important. The estimation of the rate of biochar to be applied should be based on these properties. Gray et al. (2014) even propose the designing of biochar to the specific purpose of increasing soil water retention capacity of soils.

Biochar amendment can be used as an alternative to liming with a relatively long effect, depending on the level of acidity of the soil. In some regions of Brazil, the access to residues from bioenergy production from biomass can improve the resource use efficiency of biochar as liming. In urban centres of the Brazilian Central West region, where the use of charcoal from wood is a traditional way of making barbecues generates large quantities of residues that could be used as biochar (Fig. 3 - a). If this residue was systematically collected and processed, then farmers around the city could use it. Costs with liming and mineral fertilization for vegetable production, for example, could be saved. Further, the pyrolysis of domestic organic residues could deliver production of energy and biochar, reducing environmental pollution. High technology for waste management via pyrolysis is already available (http://www.pyreg.de/machinery-en.html). Complementary, cone kilns are available to a small-scale transformation of biomass residue in biochar (Fig. 3 - b). Calories produced in the process could be used as a source of energy, such as heating for example.

Biochar can enhance soil organic matter with time once crop rotation and no tillage is adopted. The use of biochar in combination with complementary annual N fertilization and fresh organic matter (added via crop residues) seems to be the key components to build up soil organic matter in the clay soil. Liu et al. (2012a) and Schulz et al. (2013) reported the importance of combination of biochar with organic fertilization or composting biochar to improve soil fertility. Schmidt (2008) argues that biochar is not a fertilizer, but rather a nutrient carrier and a habitat for microorganisms and that, first, it has to be charged to be biologically active in order to efficiently produce its soil-enhancing properties. Interestingly, large quantities of ash and charcoal, plant and animal residues, as well as the presence of clay, evident from pieces of ceramic, are important components of the original ‘*Terra Preta de Índio*’, the fertile anthropogenic soil rich in stable C of the Amazon Region (Novotny et al. 2009). According to Schmidt (2008), there are numerous methods of activating and producing substrates similar to *Terra Preta*, as for example, the use of biochar with compost. Another successful example is the use of biochar in creating humus-rich fertile soils from waste products by Gerald Dunst in Austria (http://sonnenerde.at/).

a) b)

Figure 3. Wasted biochar in a garbage bin in Goiânia, Goiás**,** by the author in May 2014 (a) and the ‘Kon-Tiki-1 deep cone kiln’ in Switzerland, by the Ithaka Institute, July 2014, source: http://www.ithaka-institut.org/en/home (b).

Biochar might not be a good alternative for increasing rice yields on a clay soil, but might be a strategic option on a sandy soil. On less fertile sandy soils, rice yields can be increased with biochar because it acts as a prompt source of nutrients (fertilizer), such as K, and enhances the water retention capacity. On the more fertile clay soil, under no tillage with crop rotation, where rice performance will depend more on the N availability, the effect of biochar on soil chemical properties such as decreasing soil pH seems to be secondary. Further, application of biochar can decrease the water retention capacity of the clay soil, decreasing the probability of an improved rice performance under aerobic conditions. Nevertheless, on the clay soil, effects of biochar on rice blast infestation is a promising result and requires investigation (*Chapters 2* and *4*). Elad et al. (2010) and Harel et al. (2012) reported on positive effects of biochar on decreasing foliar fungal infections in tomatoes, pepper and strawberry, and as far as we know, there are no studies to investigate the effect of biochar on fungal infections in rice. Improvement of plant responses to disease can be one of the benefits gained from applying biochar to soil (Elad et al. 2011).

4. Future research challenges

Based on our findings, a number of future research challenges can be identified. Firstly, simulation studies to test whether the positive effect of biochar on increasing water retention capacity of a sandy soil can reflect on better yields under varying scenarios of weather conditions are required. Such studies can support decision making by farmers about the economic impact of biochar amendment in terms of saving water via irrigation or avoiding yield losses in rain fed systems due to water scarcity. Some initiatives to model biochar effects in APSIM model is been taken by Archontoulis et al. (2014). They modified APSIM to account biochar effects on some soil properties in Iowa cropping systems. They found that effects of biochar in plant available water and corn yields were more prominent in poor quality soils (low retention capacity and soil organic matter) than in high quality soils. Further, the amount of biochar applied had a great impact on the output of simulations, and corn yields varied along seasons, soils and cropping systems. Similarly, we also observed varying effects of biochar depending on soil type, especially regarding the soil water retention capacity (Table 1). Our newly fitted soil water retention curves at different biochar input levels can be directly added to a model like APSIM to simulate with long term weather data how much drought risks can be reduced and long term average yield increased. There are still many challenges in modelling, for example, our results show long-term effects on soil organic matter in the clay soil and no effects on N_2O emissions. It remains to be tested if models can capture these processes well. Modelling the effects of biochar amendment on water use efficiency and N dynamics in rice systems, under rain fed conditions or different irrigation regimes, for instance, is a potential work to be done.

Secondly, to test the effect of biochar on size and distribution of soil aggregates, and on the quantity and quality of C within aggregates, can clarify how biochar amendment enhances soil organic matter and how it can change the structure of clay Ferralsols along years after its application. Studies on this research field can help to elucidate the mechanisms behind C sequestration in cropping systems with biochar amendment.

Thirdly, to assess the effect of biochar on rice blast infestation under field conditions is promising and calls for further measurements in field trials properly designed to investigate this subject. Apart from water deficit, rice blast is the most important constraint for rice production in Brazil (Breseghello et al. 2011, Colombari et al. 2013). We found some results suggesting that biochar could help to safeguard the crop against infestation via increased micronutrient availability, in particular Mn.

Fourth, to test the effect of biochar with higher levels of N fertilization than the maximum used in this study might deliver different output. Perhaps biochar would have a more positive effect on rice yields on the clay soil if more mineral N fertilization was applied, avoiding a decreased N availability to the crop. However, it was not clear from our study whether there was N immobilization with biochar application. Further simultaneous investigation of the effects of biochar amendment on crop yield and soil biology under field conditions, especially on those microorganisms responsible for N dynamics in soil, is lacking. The effect of biochar on reducing the water retention capacity of the clay soil can also be a reason for a reduced N uptake by the crop. Related to the above, we recommend testing the effect of biochar with other crops than rice. Generally, biochar is likely to have a positive impact on crop yields of legumes species, because of effects on soil pH, increased availability of K and a decrease in N availability (Rondon et al. 2007**,** Nelson et al. 2011, Jeffery et al. 2011). Possibly rice benefits less due to the liming effect of biochar while rice grows relatively well in acid conditions (*Chapter 2*). Further, possibly biochar has favourable effects, but for the N immobilization, in which case rice with higher N application, leguminous crops or rice/legume crop rotations could benefit more from biochar amendment than rice mono cropping systems.

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Summary

Production of aerobic rice (*Oryza sativa* L.) in Brazil has potential for improvements. Rainfall variability and low soil fertility are the main constraints for the large yield gap in the Brazilian Savannah, where around 38% of the aerobic rice is grown. Agronomic solutions, such as improving soil fertility and providing supplementary irrigation, contribute to better productivity and improved yield stability in this region. Liming, mineral fertilization, no-tillage, crop rotation and intercropping are all practices already applied by Brazilian farmers in order to maintain the soil fertility for crop production. However, access to lime and mineral fertilizers is limited. Carbonized biomass or biochar, a local by-product of bioenergy production, could be considered as an alternative soil amendment. The biochar tested as a soil amendment in this study is a residue of the charcoal production from eucalyptus wood. Biochar is a porous, C rich material, with high K availability. Its C is pyrogenic and relatively resistant to decomposition, a desirable characteristic under tropical conditions.

This study tested the use of wood biochar as a soil amendment in two representative systems for aerobic rice production in the Brazilian Savannah: on a sandy soil under rain fed conditions and on a clay soil with supplementary sprinkler irrigation. The wood biochar was applied once, at the beginning of the field trials. A rate of 0, 8, 16 and 32 Mg ha^{-1} of wood biochar milled to pass a 2-mm sieve was incorporated into 0-15 cm soil layer in plots of 40 m^2. The mineral N fertilization was applied annually at a rate of 0, 30, 60 and 90 kg N ha^{-1} and subdivided in two or three applications: at sowing and as one or two top dressings during the growing season. All plots received the same rate of mineral fertilization (K-P) applied annually according to chemical soil analysis and the demand by the crop. Experimental measurements were taken for four to five consecutive cropping seasons. Effects of biochar and N fertilization on yields and soil chemical and physical properties were measured annually.

In *Chapter 2* we presented the short term effects, at 0.5 year after biochar application, on chemical properties in the clay soil and its impact on rice growth, yield and yield components. Biochar improved some of the soil chemical properties of the clay soil. Specifically, it lead to a decrease in soil acidity and an increase in nutrient availability. However, rice yield was not affected by biochar application, as a negative effect of biochar on rice growth was counterbalanced by a positive effect on harvest index. Of relevance was the positive effect of biochar on decreasing the number of panicles infested by rice blast

(*Magnaporthe grisea*). Highest grain yield (around 3 Mg ha^{-1}) was obtained with 46 kg N ha^{-1}, regardless of the biochar rate applied. Our results indicated that biochar could have short-term temporary negative effects on rice growth, probably due to a decrease in N availability to the crop. Such a decrease in N availability to the crop can be caused by N immobilization, increased N losses via N_2O-N fluxes or even by a decrease in soil water availability.

In *Chapter 3*, the role of biochar on increasing the plant available water in the low fertile sandy soil was investigated at 2 and 3 years after application. Biochar had an effect on overall porosity of the sandy soil, and water retention capacity increased linearly with biochar rate. The increment in rice stress free available water means an additional safeguard of approximately four days to withstand periods without rainfall under the dry prone conditions of the Brazilian Savannah. However, biochar had no effect on rice yield in the third and fourth season after application. Previous research, in the first two seasons after biochar application, revealed that rice yield increased linearly with biochar rate. In this study, the four seasons that were monitored on the sandy soil differed in terms of the amount of rainfall received: in two of the seasons (2 and 3 years after biochar application, respectively) the amount of rainfall was high ($\geq$ 650 mm; twice the amount during the critical period for rice production in the two previous seasons). This is the most probable cause for the divergent effect of biochar on rice yield on the sandy soil. Additionally, we validated a novel approach to model and compare soil water retention curves that can contribute to more precise estimates of this environmentally important soil hydraulic function. Generally, fitting of curves is performed for isolated treatments, without accounting for experimental structure. The isolated treatment-specific model fitting has three main disadvantages: first, comparison of the curves between treatments via formal statistical tests is not possible due to the absence of an error structure that accounts for overall variance within treatments; secondly, autocorrelations among random errors of moisture measurements taken in the same sample unit (the cylinder) under different matric potentials are ignored, leading to incorrect quantification of model uncertainty; and thirdly, the spatial variability, likely to be high under field conditions, cannot be fully accounted for. In *Chapter 3* we developed and tested nonlinear mixed models to overcome these disadvantages.

In *Chapter 4* we demonstrated the impact of biochar amendment on soil water retention capacity of the clay soil, using the methods developed in *Chapter 3*. We showed that at 1.5 and 2.5 years after biochar application, the soil water retention capacity of the clay soil decreased with biochar rate, especially at matric potential between -6 and -100 kPa, i.e., the matric potential at which water is free available and the rice crop is not exposed to water limitations. We also showed the residual effects of biochar, at 1.5, 2.5 and 3.5 years after application, on chemical properties and rice yield. Effects of biochar on decreasing soil acidity remained up to at least 3.5 years after application. Soil organic matter increased linearly with biochar rate at 2.5 and 3.5 years after application, the last seasons assessed. Further, rice yield varied among seasons. Rice yield decreased linearly with biochar rate, regardless of the N applied, at 1.5 year after biochar application. At 2.5 years after application, rice yield increased linearly with biochar rate, but only if more than 60 kg N ha^{-1} was applied. Finally, at 3.5 years after biochar application, no effect of biochar on rice yield was observed. Our results indicate that the negative effect of biochar amendment on rice growth and yield at 1.5 and 2.5 year after its application is most probably a result of a decrease in water availability and, consequently N availability, to the crop.

In *Chapter 5* we showed the impact of 32 Mg ha^{-1} biochar, without or in combination with 90 kg N ha^{-1}, on N_2O-N fluxes along four cropping seasons, from immediately after application to 2.5 years later. The annual mineral N fertilization was the main factor for enhanced N_2O-N fluxes along the seasons under assessment. Biochar, on the other hand, had no effect on N_2O-N fluxes along the four cropping seasons. Therefore, the hypothesis, proposed based on the findings in *Chapter 2*, that the negative effect of biochar on rice growth at 0.5 year after application was a result of increased N losses via N_2O-N fluxes is unlikely. Effects of biochar on chemical and physical properties of the clay soil indicates that there is an ongoing interaction process of biochar with the soil matrix. Further assessment of the soil organic matter content and physical structure, such as the size distribution of aggregates in the clay soil, are required to elucidate the role of biochar in sequestrating C from atmosphere via enhancement of soil fertility.

In *Chapter 6* we discuss the main findings of this study, the methodological and statistical approaches and the contribution of biochar to sustainable intensification of farming systems in the Brazilian Savannah. This study is an innovative contribution to biochar research, because it was designed to evaluate the effect of biochar under real farming conditions, with feasible amounts of biochar and mineral N fertilization. The main constraints for designing such type of field experiments are related to operational features, such as irrigation, sowing and fertilization events. The use of mixed models allowed us to overcome constraints related to the lack of randomization of mineral N fertilization, the high spatial variability, and to account for potential correlations among repeated measurements taken in space and time. The use of this approach allows for a more precise estimate of parameter values. Therefore, the study makes valuable methodological as well as practical contribution to biochar research. The main findings of this study, also summarized in Table 1 of *Chapter 6*, are that biochar effects were more positive on water retention capacity of the sandy soil, soil organic matter content of the clay soil and on soil acidity in both soil types. Therefore, biochar is a suitable soil amendment for improvement of soil chemical and physical properties of these weathered soils of the Brazilian Savannah. Additionally, effects on rice yield can vary among seasons and with the amount of biochar and mineral N fertilization applied. Items that deserve further study, like the suppressive effect of biochar on rice blast disease, were also identified.

The main conclusion about the impact of wood biochar amendment in aerobic rice systems of the Brazilian Savannah is that biochar can improve soil fertility via reduction of soil acidity and increased nutrient availability. Application of wood biochar and mineral N fertilization in the rates used in the current study were insufficient to ensure increases in aerobic rice yield. Obviously, the best opportunities for increases in rice yield are found on sandy soils. Economic assessment of the biochar as a substitute of lime should reveal the prospects of the use of this by-product as a soil amendment by farmers in this region. The findings obtained in the this study show the great importance of longer-term field experimentation that does consider effects on both chemical and physical components of soil fertility, when the aim is to estimate the value of biochar for present-day farming.

Samenvatting

De opbrengst van aerobe rijst in Brazilië is voor verbetering vatbaar. In de Braziliaanse Savannah, waar rond 38% van de Braziliaanse aerobe rijst verbouwd wordt, zijn lage bodemvruchtbaarheid en onregelmatige regenval de belangrijkste opbrengst-beperkende factoren. Landbouwkundige oplossingen, zoals het verbeteren van de bodemvruchtbaarheid en beregening, kunnen bijdragen aan hogere en stabielere opbrengsten in deze regio. Boeren passen al veel teeltmaatregelen toe om de bodemvruchtbaarheid op peil te houden, zoals bekalking, bemesting, het afzien van grondbewerking, vruchtwisseling en mengteelt. Kalk en kunstmeststoffen zijn evenwel niet altijd makkelijk verkrijgbaar. Om deze reden is onderzocht in hoeverre biochar als een alternatieve bodemverbeteraar gebruikt kan worden. Biochar is de verzamelnaam voor het koolstofrijk, stabiel en vast eindproduct van pyrolyse, waarbij organisch materiaal op hoge temperatuur en zonder externe toevoer van zuurstof wordt gekraakt. In de Braziliaanse Savannah is biochar beschikbaar als lokaal bijproduct van bio-energie productie. De in deze studie geteste biochar was een restproduct van de houtskoolproductie uit eucalyptus hout. Het materiaal is poreus en heeft hoge gehaltes aan koolstof (C) en kalium (K). De koolstof is moeilijk afbreekbaar; een nuttige eigenschap onder tropische omstandigheden.

Dit proefschrift beschrijft de resultaten van een onderzoek naar de gevolgen van toediening van biochar op twee representatieve rijst-productiesystemen in de Braziliaanse Savannah: op een zandgrond zonder irrigatie en op een kleigrond met irrigatie. Vier hoeveelheden biochar werden gebruikt: 0, 8, 16 en 32 Mg ha^{-1}. De biochar werd vooraf vermalen en door een zeef met een maaswijdte van 2 mm gewerkt en vervolgens eenmalig, aan het begin van het experiment, ingewerkt in de bovenste 15 cm van de bouwvoor van veldjes ter grootte van 40 m^2. Jaarlijks werden vier hoeveelheden stikstof (N) in de vorm van kunstmest gegeven: 0, 30, 60 en 90 kg N ha^{-1}. Een deel van de kunstmest werd bij het zaaien gegeven, de rest in de loop van het groeiseizoen. Elk veldje werd ook bemest met een identieke hoeveelheid kalium en fosfaat, waarbij de hoeveelheid werd afgestemd op een bodemchemische analyse en de verwachte vraag van het gewas. De proefvelden werden gedurende 4 tot 5 groeiseizoenen gevolgd. Tijdens deze seizoenen werden rijstopbrengst en bodemfysische en bodemchemische eigenschappen gemeten.

In *Hoofdstuk 2* presenteren we de korte termijn effecten op de kleigrond, ongeveer een half jaar na de biochar gift. Biochar verbeterde sommige bodemchemische kenmerken van de

kleigrond. Het maakte de bodem minder zuur en vergrootte de beschikbaarheid van nutriënten. Biochar had evenwel geen effect op de rijstopbrengst, omdat een hogere oogst index te niet werd gedaan door een lagere biomassa productie. Volgens de regressie modellen zou de hoogste opbrengst (3 Mg ha^{-1}) behaald worden met een N gift van 46 kg N ha^{-1}, ongeacht de biochar dosis. Opvallend was dat biochar leidde tot een vermindering van het aantal door de brandvlekkenziekte (*Magnaporthe grisea*) aangetaste halmen. De resultaten laten zien dat biochar op korte termijn een negatief effect op groei kan hebben, waarschijnlijk veroorzaakt door een lagere N beschikbaarheid voor het gewas. Een dergelijke verminderde N beschikbaarheid zou veroorzaakt kunnen zijn door N immobilisatie, toegenomen N verliezen door N_2O-N fluxen of door een verminderde hoeveelheid beschikbaar bodemvocht.

In *Hoofdstuk 3* werd het effect van biochar op voor de plant beschikbaar bodemvocht onderzocht, rond 2 en 3 jaar na de biochar gift. Dit onderzoek werd uitgevoerd op de minder vruchtbare zandgrond. Biochar verhoogde de algehele porositeit van de zandgrond, en de maximale hoeveelheid vocht die in de bodem kan worden opgeslagen (bodemvochtcapaciteit) nam lineair toe met de hoeveelheid biochar. De toename in bodemvochtcapaciteit was zodanig dat de rijst ongeveer 4 dagen extra droogte zou kunnen doorstaan. Dat is van belang in de Braziliaanse Savannah, waar tijdens het natte seizoen kortere periodes van droogte een groot probleem kunnen zijn. Onderzoek in de eerste twee seizoenen na toediening van biochar liet zien dat de rijstopbrengst lineair toenam met biochar. In het derde en vierde seizoen, de seizoenen waarin dit onderzoek zich afspeelde, had biochar echter geen effect. Waarschijnlijk kwam dit doordat in de laatste twee seizoenen de hoeveelheid regen tijdens het groeiseizoen exceptioneel hoog was ($\geq$ 650 mm tijdens de kritische periode van de groei, twee maal zo hoog als in de eerste seizoenen). In dit hoofdstuk ontwikkelden we bovendien een nieuwe methode om vocht-retentiecurves (pF-curves) te modelleren en vergelijken. Normaal gesproken worden dergelijke curves voor elke behandeling apart bepaald. Apart voor elke behandeling de curves bepalen heeft evenwel drie belangrijke beperkingen. Ten eerste is het niet mogelijk om, rekening houdend met de structuur van het experiment, via formele statistische toetsen de curves te vergelijken. Deze beperking leidt tot een tweede beperking: door de curves apart voor elk monster te bepalen kan geen rekening gehouden worden met ruimtelijke variabiliteit, die groot kan zijn bij veldproeven. Een derde beperking is dat normaal gesproken geen rekening wordt gehouden met autocorrelatie in de metingen binnen de monster eenheid (de cilinder), waardoor fouten ontstaan in de berekening van model onzekerheid. Voor *Hoofdstuk 3* ontwikkelden we niet-lineare, gemengde modellen om deze beperkingen het hoofd te bieden.

In *Hoofdstuk 4* onderzochten we de gevolgen van biochar voor vocht-retentiecurves van de kleigrond, gebruik makend van de in *Hoofdstuk 3* ontwikkelde methode. We toonden aan dat 1.5 en 2.5 jaar na de biochar gift de bodemvochtcapaciteit afnam door toedoen van biochar en dan vooral in matrix potentialen tussen -6 and -100 kPa. Dat is juist de range waarin rijst zonder veel problemen vocht kan opnemen. Biochar had gedurende het hele experiment, tot 3.5 jaar na toediening van de biochar, effect op enkele bodemchemische kenmerken en op de rijstopbrengst. De bodem werd blijvend minder zuur en in de laatste twee seizoenen werd een lineaire verhoging van het organische stof gehalte in de bodem waargenomen. De effecten van biochar op de rijstopbrengst veranderden met de tijd. Een half jaar na de biochar gift was er geen effect op de opbrengst (*Hoofdstuk 2*). Anderhalf jaar na de biochar gift leidde biochar tot opbrengst reductie. Na 2.5 jaar nam de opbrengst lineair toe met biochar, maar alleen bij een N-gift van ten minste 60 kg ha^{-1}. En tenslotte, 3.5 jaar na de biochar gift, was er geen effect op opbrengst. De resultaten suggereren dat de negatieve effecten 1.5 en 2.5 jaar na de biochar gift naar alle waarschijnlijkheid werden veroorzaakt doordat minder vocht, en daarmee minder stikstof, beschikbaar waren voor het gewas.

In *Hoofdstuk 5* onderzochten we de effecten van 32 Mg ha^{-1} biochar met en zonder 90 kg N ha^{-1} op N_2O emissies op de klei bodem, van direct na toediening tot 2.5 jaar na inwerken van biochar in de bodem. Meer stikstof leidde tot meer N_2O emissies, terwijl biochar geen effect had. Om die reden is de hypothese, geformuleerd op basis van de in *Hoofdstuk 2* gerapporteerde resultaten, namelijk dat de negatieve effecten van biochar op de groei van rijst veroorzaakt zouden zijn door verhoogde N_2O emissies, uiterst onwaarschijnlijk. Effecten van biochar op de fysische en chemische eigenschappen van de bodem doen vermoeden dat er een continue interactie is tussen biochar en de bodemmatrix. Verder onderzoek naar de gevolgen van biochar op bodem organische stof en de fysieke structuur, zoals de grootte en de verdeling van bodemaggregaten, is nodig om verder inzicht te krijgen in de rol van biochar in het vastleggen van koolstof (C) uit de atmosfeer.

In *Hoofdstuk 6* bespreken we de belangrijkste uitkomsten van het onderzoek, de methodische en statistische benaderingen en de mogelijke bijdrage van biochar aan duurzame intensivering van bedrijfssystemen in de Braziliaanse Savannah. Nieuw aan deze studie was dat deze studie is uitgevoerd onder veldomstandigheden en met realistische hoeveelheden biochar en stikstof-kunstmest. Dit in tegenstelling tot vele laboratorium studies waarin mogelijk relevante veldcondities niet optreden en waar vaak praktisch onrealistisch grote hoeveelheden biochar gebruikt worden. De grootste belemmeringen bij het opzetten en uitvoeren van veldproeven zoals beschreven in dit proefschrift zijn van operationele aard en hebben betrekking op irrigatie, zaaien en bemesting. Gebruik makend van gemengde modellen (mixed models), een statistische techniek, kon rekening gehouden worden met onvolkomenheden in de proefopzet. Zo kon om praktische redenen de N-bemesting niet volledig random worden toegepast en was er sprake van een hoge mate van ruimtelijke variatie. Ook kon dankzij de gemengde modellen rekening gehouden worden met correlatie tussen herhaalde metingen aan één en hetzelfde monster (autocorrelatie). Zodoende konden bepaalde parameters preciezer geschat worden en vooral ook de onzekerheid beter gekwantificeerd. Dit proefschrift biedt hiermee een waardevolle methodische en praktische bijdrage aan het onderzoek gericht op biochar. De belangrijkste uitkomsten, samengevat in Tabel 1 uit *Hoofdstuk* 6, zijn dat biochar op zandgrond een positief effect had op bodemvochtcapaciteit, op kleigrond een positief effect had op bodem organische stof, terwijl op beide bodems de verzuring werd verminderd. Daarmee is biochar een geschikte bodemverbeteraar van zowel fysische als chemische eigenschappen van de zwaar verweerde bodems in de Braziliaanse Savanna. Het effect op de rijstopbrengst was niet consistent, sterk seizoensgebonden en afhankelijk van de hoeveelheid toegediende biochar en stikstof-kunstmest. Daarnaast werden andere onderwerpen voor verdere studie, zoals het onderdrukkende effect van biochar op de brandvlekkenziekte, geïdentificeerd.

De belangrijkste conclusie over het nut van biochar voor aerobe rijstproductie in de Braziliaanse Savannah is dat biochar de bodemvruchtbaarheid kan verhogen door verhoging van de zuurgraad en een verhoogde beschikbaarheid van nutriënten. De hoeveelheden biochar en stikstof-kunstmest in deze studie waren echter te laag om daadwerkelijk de opbrengst te verhogen. Duidelijk is dat toepassing van biochar op zandgrond de beste mogelijkheid biedt voor een verhoging van de rijstopbrengst. Economisch analyse zou moeten uitwijzen of biochar voor de boeren in deze regio een aantrekkelijk alternatief kan zijn voor bekalking. De uitkomsten van deze studie laten zien hoe belangrijk langdurige veldproeven zijn voor onderzoek naar de waarde van biochar als bodemverbeteraar in de huidige landbouwpraktijk.

Sumário

Existe um potencial para o aumento da produtividade do arroz (*Oryza sativa* L.) de terras altas no Cerrado brasileiro, onde são cultivados 38% dessa cultura. A variabilidade pluviométrica e a baixa fertilidade do solo são consideradas as principais causas da oscilação de produtividade do arroz de terras altas no Cerrado. Soluções agronômicas, como a melhoria da fertilidade do solo e irrigação suplementar, contribuem para o aumento e estabilidade da produtividade nessa região. Calagem, adubação mineral, plantio direto, rotação e consorciação de culturas são práticas já utilizadas pelos agricultores brasileiros no sentido de manter a fertilidade do solo para produção agrícola. Porém, para a maioria dos agricultores o acesso a essas práticas de manejo é limitado. A biomassa carbonizada ou biochar, um subproduto local da produção de energia a partir de biomassa, pode ser considerada como um condicionador de solo alternativo. Nesse estudo o biochar testado como condicionador de solo é um resíduo da produção de carvão vegetal de eucaliptos, denominado popularmente como pó fino de carvão. Esse material é poroso, rico em C e com alta disponibilidade de K. O seu C é pirogênico e relativamente resistente à decomposição, uma característica desejada em condições tropicais.

Nesse estudo testou-se o uso de biochar como condicionador de solo em dois sistemas agrícolas representativos da produção de arroz de terras altas no Cerrado, sendo um sistema em solo arenoso (Plintosolo), sem irrigação suplementar, e o outro em solo argiloso (Latossolo) com irrigação suplementar via pivô central. O biochar foi aplicado apenas uma vez, no estabelecimento dos campos experimentais. Utilizaram-se quatro doses de biochar: 0, 8, 16 e 32 Mg ha^{-1}. Esse material foi moído, passado em peneira de 2 mm e incorporado na profundidade de 0-15 cm do solo em parcelas de 40 m^2. A adubação com N mineral foi aplicada anualmente nas doses de 0, 30, 60 e 90 kg N ha^{-1} e subdivididas em três aplicações, no plantio e em duas coberturas no florescimento da cultura. Todas as parcelas receberam a mesma dose de K e P que foram aplicados anualmente de acordo com análise química do solo e a demanda da cultura. O experimento foi conduzido por até cinco safras consecutivas. Os efeitos do biochar e da adubação com N mineral sobre a produtividade do arroz e as propriedades químicas e físicas do solo foram medidos anualmente.

No *Capítulo 1* foi apresentada uma revisão sobre o estado da arte da pesquisa em biochar como condicionador de solo em sistemas agrícolas, além da caracterização edafoclimática e da discussão sobre as dificuldades para a produção de arroz de terras altas na região onde o estudo foi realizado. No *Capítulo 2* foram discutidos os efeitos do biochar em

curto prazo, 0,5 ano após a aplicação, sobre as propriedades químicas do solo argiloso e seu impacto no crescimento, desenvolvimento e produtividade do arroz e seus componentes. O uso de biochar melhorou algumas propriedades químicas do solo. Especificamente, diminuiu a acidez do solo e aumentou a disponibilidade de nutrientes. Entretanto, isso não foi suficiente para afetar a produtividade do arroz, já que a redução da área foliar e da massa da matéria seca aos 75 dias após plantio foi contrabalanceado por um efeito positivo sobre o índice de colheita para as doses de biochar aplicadas. Um aspecto relevante foi a redução do número de panículas infestadas por brusone (*Magnaporthe grisea*) proporcional ao aumento das doses de biochar aplicadas. A maior produtividade (aproximadamente 3 Mg ha^{-1}) foi obtida com 46 kg N ha^{-1}, independentemente da dose de biochar aplicada. Nossos resultados indicam que, no curto prazo, o uso de biochar teve um efeito negativo sobre o crescimento do arroz, provavelmente devido à redução da disponibilidade de N para a cultura. Essa redução pode estar relacionada à imobilização do N, aumento das perdas N por fluxos de N_2O-N ou devido a uma diminuição na água disponível do solo.

No *Capítulo 3* foi investigado o efeito do uso do biochar sobre a água disponível para planta em um solo arenoso após 2 e 3 anos da aplicação. O uso de biochar alterou a porosidade total do solo e aumentou linearmente a capacidade de retenção de água para as doses de biochar aplicadas. Esse aumento na água disponível para o arroz significa um incremento adicional de aproximadamente dois dias para suportar períodos de estiagem durante o crescimento da cultura no Cerrado. Entretanto, esse incremento não proporcionou um aumento na produtividade do arroz na terceira e quarta safras após aplicação do biochar. Estudo prévio, nas duas primeiras safras após aplicação do biochar, revelou que a produtividade do arroz aumentou linearmente com as doses de biochar. Entretanto, no presente estudo observou-se uma variação na precipitação acumulada para as quatro safras em solo arenoso: em duas das safras (2 e 3 anos após aplicação do biochar) a quantidade de precipitação foi maior ($\geq$ 650 mm), ou seja, o dobro da quantidade durante o período crítico para produção de arroz do que observado nas duas primeiras safras. Esta diferença na precipitação acumulada é provavelmente a causa dos efeitos divergentes do uso de biochar sobre a produtividade de arroz em solo arenoso. Adicionalmente, nós validamos um novo modelo para ajustar e comparar as curvas de retenção de água do solo, que pode contribuir para estimativas precisas desta função hidráulica, que é ambientalmente importante. Geralmente, o ajuste de curvas é feito para tratamentos isolados, sem considerar a estrutura do experimento. O ajuste isolado das curvas, ou seja, para cada tratamento específico, apresenta três desvantagens: a comparação das curvas entre tratamentos por meio de testes estatísticos formais não é possível devido à ausência de uma estrutura de erro que considere a variância total dentro de tratamentos; autocorrelações entre erros de medidas de umidade tomadas numa mesma amostra (o cilindro) em diferentes potenciais matriciais são ignoradas, levando à quantificação incorreta de incertezas no modelo; e, a variabilidade espacial, geralmente alta em condições de campo, não pode ser totalmente considerada. Nesse capítulo nós desenvolvemos e testamos modelos mistos não lineares para superar estas desvantagens.

Já no *Capítulo 4* foi demonstrado o impacto do uso de biochar sobre a capacidade de retenção de água do solo argiloso, por meio do método desenvolvido no *Capítulo 3*. Nesse capítulo mostramos que aos 1,5 e 2,5 anos após aplicação do biochar, a capacidade de retenção de água no solo argiloso diminuiu com as doses de biochar, especialmente para um potencial mátrico entre -6 e -100 kPa, isto é, o potencial mátrico no qual a água está disponível, não havendo deficiência hídrica. O efeito residual do uso do biochar, aos 1,5, 2,5 e 3,5 anos após aplicação sobre as propriedades químicas do solo e produtividade do arroz também é abordado. A diminuição da acidez do solo devido à aplicação do biochar foi observado até 3,5 anos após sua aplicação. Houve um incremento linear no teor de matéria orgânica no solo para as doses de biochar aos 2,5 e 3,5 anos após aplicação. Além disso, a produtividade do arroz variou entre as safras. A produtividade diminuiu linearmente com as doses de biochar, independente do N mineral aplicado, aos 1,5 anos após aplicação do biochar. Aos 2,5 anos após aplicação, a produtividade do arroz aumentou linearmente com as doses de biochar, entretanto isso foi observado somente quando se aplicou mais de 60 kg N ha^{-1}. Finalmente, aos 3,5 anos após aplicação não houve efeito do uso de biochar sobre a produtividade do arroz. Nossos resultados indicam que o efeito negativo do biochar sobre o crescimento e a produtividade do arroz aos 1,5 e 2,5 anos após sua aplicação é provavelmente devido à redução de água disponível no solo e, consequentemente, do N disponível para a cultura.

No *Capítulo 5* foi estudado o impacto de 32 Mg ha^{-1} biochar, sem N mineral ou em combinação com 90 kg N ha^{-1}, sobre os fluxos de N_2O-N no período de quatro safras, imediatamente após aplicação do biochar até 2,5 anos de sua aplicação no solo argiloso. A adubação anual de N mineral foi o principal fator para o aumento dos fluxos de N_2O-N durante as safras monitoradas. O uso de biochar, em compensação, não teve efeito sobre os fluxos de N_2O-N durante as quatro safras. Portanto, a hipótese embasada nos resultados do *Capítulo 2*, de que o efeito negativo do biochar sobre o crescimento do arroz ao 0,5 ano após sua aplicação seria devido ao aumento das perdas de N por fluxos de N_2O-N é improvável. Efeitos do biochar sobre as propriedades químicas e físicas do solo argiloso indicam que o processo de interação entre biochar e a matriz do solo é dinâmico. Além disso, futuras avaliações do conteúdo de matéria orgânica e da estrutura física do solo, como agregação, são necessárias para elucidar o papel do biochar no sequestro de C da atmosfera via aumento da fertilidade do solo.

No *Capítulo 6* foram discutidos os principais resultados, a abordagem estatística e metodológica e a contribuição do uso de biochar para intensificação sustentável de sistemas agrícolas no Cerrado. O presente estudo é uma contribuição inovativa para pesquisa em biochar porque foi delineado para avaliar o efeito do biochar em condições reais de cultivo, com quantidades factíveis de biochar e adubação nitrogenada. As principais limitações para o delineamento de tal tipo de experimento de campo estão relacionadas às características operacionais, como os eventos de irrigação, plantio e fertilização. O uso de modelos mistos nos permitiu superar as limitações relacionadas à incompleta randomização da aplicação do N mineral, a alta variabilidade espacial e considerar possíveis correlações entre medidas repetidas no espaço e no tempo. O uso desta abordagem permitiu uma estimativa precisa dos parâmetros. Portanto, esse estudo traz uma contribuição metodológica para pesquisas sobre o valor agronômico do biochar. Os principais resultados desse estudo, também sintetizados na Tabela 1 do *Capítulo 6,* são de que o uso de biochar aumenta a capacidade de retenção de água do solo arenoso, o conteúdo de matéria orgânica no solo argiloso e reduz a acidez em ambos os solos. Portanto, pode-se considerar o biochar como um condicionador de solo apropriado para a melhoria das propriedades químicas e físicas dos solos intemperizados do Cerrado. Concomitantemente, os efeitos sobre a produtividade do arroz podem variar entre as safras e de acordo com as doses de biochar e N mineral aplicadas. Itens que merecem futura investigação, como o efeito supressivo do biochar sobre brusone, também foram identificados.

A principal conclusão sobre o impacto do uso de biochar como condicionador de solo em sistemas de produção de arroz de terras altas no Cerrado é que o mesmo pode melhorar a fertilidade do solo por meio da redução da acidez e do aumento da disponibilidade de nutrientes. Aplicação de biochar e N mineral em doses usadas no presente estudo foram insuficientes para garantir o aumento da produtividade de arroz de terras altas. Obviamente, as melhores oportunidades para o incremento da produtividade do arroz são encontradas nos solos arenosos. A avaliação econômica do biochar como um substituto à calagem deve revelar os prospectos para o uso deste resíduo como condicionador de solo por agricultores no Cerrado. Os resultados obtidos neste estudo mostram a importância de campos experimentais de longa duração que considerem os efeitos em ambos componentes, químicos e físicos da fertilidade do solo, quando o objetivo é estimar o valor do uso de biochar na agricultura atual.

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"This life counts, and don't be fooled, there's just one!
Two lives, which would be good, nobody can claim to have (...).
Life isn't a game, friend. Life is the art of the encounter.
Even though there might be so much discord (dis-encounter) in this life (...).
Put a little bit of love in your life, like in your samba!"

(Samba da Bênção by Vinícius de Moraes and Baden Powell, 1967)

Thesis organization

This thesis is the product of collaborative research between the School of Land and Food at the University of Tasmania, Australia, and the Centre for Crop Systems Analysis (CSA) at Wageningen University (WU), and is part of a double degree finalized with a defence in Wageningen, The Netherlands. The examination procedure was jointly administered by the two universities and conform the regulations of both.

List of Publications

Carvalho, M. T. M., Maia, A. H. N., Madari, B. E. M., Bastiaans, L., van Oort, P. A. J., Heinemann, A. B., da Silva, M. A. S., Petter, F. A. and Meinke, H., 2014. Biochar increases plant available water in a sandy soil under an aerobic rice cropping system. Solid Earth, 5: 939-952.

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PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Writing of project proposal (4.5 ECTS)

- Towards sustainable production of aerobic rice in the Brazilian Savannah: investigating the potential of carbonized biomass as soil amendment

Post-graduate courses (5.6 ECTS)

- Soil ecology: taking global issues underground; PE&RC, SENSE, RSEE (2010)
- The art of modelling; SENS, PE&RC (2010)
- Mixed linear models; PE&RC (2012)

Laboratory training and working visits (4.5 ECTS)

- The use of biochar as a soil amendment; Tasmanian Institute of Agriculture / University of Tasmania (2012 / 2013)

Deficiency, refresh, brush-up courses (3 ECTS)

- Designing sustainable cropping systems (2011)
- Basic statistics (2012)

Competence strengthening / skills courses (3 ECTS)

- Information literacy PhD including EndNote; WUR Library (2010)
- Techniques for writing and presenting a scientific paper; Wageningen Graduate Schools (2011)
- Dutch for employees I; Language Services of WUR (2011)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.4 ECTS)

- PE&RC Weekend (2010, 2013)
- PE&RC Day: innovation for sustainable, what are the neighbours doing? (2011)
- Thee WE DO day (2012)

Discussion groups / local seminars / other scientific meetings (4.4 ECTS)

- Seminar prior PhD thesis defense: adaptation to climate related risks in managed river basins: diversifying land use and water management activities to adapt to climate related risks in the Netherlands and Hungary; WUR (2010)
- Introduction to field trials on biochar at Embrapa Rice and Beans (2010)
- Seminar on biochar: the terra Preta Program; WUR (2010)
- The first meeting of the LabMic of the Institute of Physics; Federal University of Goiás (2011)
- Discussion group on biochar (2011)
- Seminar on biochar: the terra Preta Program; WUR (2011)
- Seminar on intensive Rice Cultivation ; WUR (2012)
- Seminar on food in Africa; WUR (2012)
- Discussion group on scientific research in agriculture; UTAS (2013)

- Seminar on sustainable intensification of agriculture vs agroecology ; SIAS (2013)
- Seminar on agroecology: the example of Brazil; SIAS (2014)
- Seminar on scientific basis of agroecology; WUR (2013)
- Seminar on biochar: the terra Preta Program; WUR (2013)
- Seminar on publication about political aspects of biochar research in Brazil; WUR (2013)
- Seminar on biochar: hype or hope?; WUR (2013)

International symposia, workshops and conferences (8.9 ECTS)

- The Climate Food and Farming (CLIFF) network; Nairobi, Kenia (2011)
- The biobased economy, “biochar: the soil is the limit”; WUR (2012)
- The Joint SSA and NZSSS soil science conference; Hobart, Tasmania, Australia (2012)
- The Tropentag; Göttingen University, Germany (2012)
- The X Brazilian Meeting in Humic Substances (X EBSH) at Embrapa Rice and beans; Brazil (2013)

Lecturing / supervision of practical‘s / tutorials (0.3 ECTS)

- Research Methods in Crop Science (2014)

Curriculum vitae

Márcia Thaís de Melo Carvalho was born in Conceição do Araguaia, Pará State, Brazil, on 10 May 1980. There, she attended the primary school at *Sossego da Mamãe* and at *Ministro Jarbas Passarinho* School (Bradesco Foundation). In 1991, she moved with her family to Altamira, Pará State, where she attended school provided by the Industrial Social Service (SESI). One year later, in 1992, she moved with her family to Anápolis, Goiás State, where she finished her primary education at *São Francisco de Assis* School. In 1996, she moved to Goiânia, capital of Goiás State, where she finished her secondary education at *Visão* College and commenced a Bachelor of Sciences (Agronomy) degree at the Federal University of Goiás (UFG) in 1997, graduating in 2002. During her internship at the Association for Organic Agriculture of Goiás (ADAO/GO) as part of her bachelor studies, she gained practical farming experience related to urban agriculture, vegetable production, fair trade, farming association and cooperation, greenhouse and field crops production. For her master thesis, she investigated the influence of cover crops and green manure on soil chemical and physical properties and on corn (*Zea mays*) growth and yield in a two-year field experiment from 2002/2003 to 2003/2004. In 2005, she was invited to teach Natural Sciences at the State University of Pará in her hometown, where she was also involved in projects of environmental education and development of education and smallholder agriculture in rural areas. In 2008, the Brazilian Agricultural Research Corporation, Embrapa Rice and Beans, hired her to act as a researcher on the impact of agriculture systems on climate change. In April 2010, Embrapa gave her the license and a scholarship to assume a position as a PhD student during four years at the Centre for Crop Systems Analysis of Wageningen University. During her PhD she conducted detailed field experiments, laboratory analysis of plant performance and soil properties, greenhouse gas emission monitoring and developed new statistical approach to investigate the impact of wood biochar as a soil amendment in aerobic rice systems on two sites in the Brazilian Central West region. She also actively engages with climate change mitigation and biochar researchers through the Climate Food and Farming Research Network (CLIFF), an international research network that links researchers and doctoral students working on climate change mitigation in small-scale farming and food systems.

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